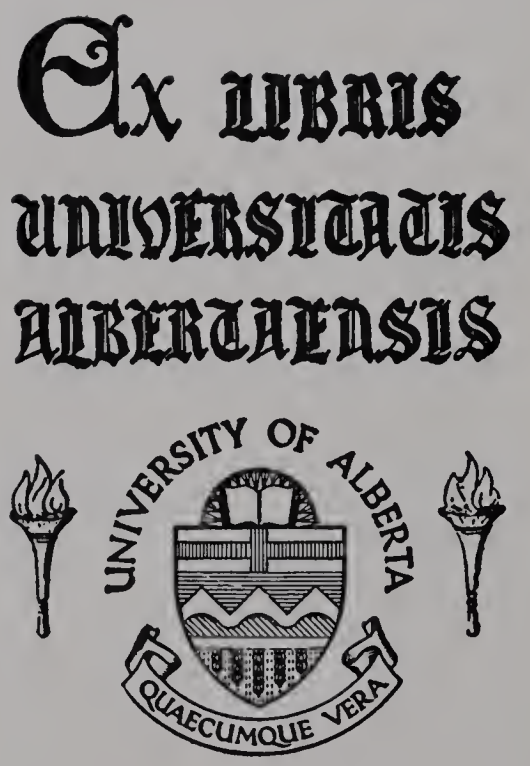

University of Alberta
Printing Department

THE UNIVERSITY OF ALBERTA

A STUDY OF THE CONJUNCTIVAL MICROVASCULATURE IN DIABETICS AND NON-DIABETICS

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES
IN PARTIAL FULFILMENT OF THE REGULATIONS FOR
THE DEGREE OF MASTER OF SCIENCE (MEDICINE)

FACULTY OF MEDICINE

by

MARTIN H. ATKINSON

May, 1966

APPROVAL SHEET

UNIVERSITY OF ALBERTA

FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "A Study of the Conjunctival Microvasculature in Diabetics and Non-Diabetics", submitted by Martin H. Atkinson in partial fulfilment of the requirements for the Degree of Master of Science (Medicine).

ABSTRACT

The conjunctival microvasculature was studied in fifty-seven diabetics and sixty-six non-diabetics. Both samples of subjects fulfilled a number of strict criteria before they were accepted for this study.

The temporal aspect of the bulbar conjunctiva of the right eye in each subject, both diabetic and non-diabetic, was photographed. Three photographs, containing at least one vessel pair, were taken in the region midway between the limbus and outer canthus.

The resulting negatives were projected on to a screen located a fixed distance from the slide. Measurements were taken of arteriolar and venular diameters. These measurements were then converted to a measure of the vessel's cross-sectional area.

Three comparisons were made between the two samples, using the Standard Error of the Difference between Means. The three comparisons were:

(1) -Mean arteriolar cross-sectional areas.

(2) -Mean venular cross-sectional areas.

(3) -Mean V/A ratios

A fourth comparison was made between the V/A ratios of two vessel pairs in the same individual, both diabetic

and non-diabetic, using the Wilcoxon Test for the Paired Case.

Within the limits of this study, the following conclusions have been made.

1. There is no significant difference between mean arteriolar cross-sectional areas of diabetics and non-diabetics.

2. There is no significant difference between mean venular cross-sectional areas of diabetics and non-diabetics.

3. There is no significant difference between mean V/A ratios of diabetics and non-diabetics.

4. There is no significant difference between mean V/A ratios of two vessel pairs in the same individual in the diabetic sample.

5. There is no significant difference between mean V/A ratios of two vessel pairs in the same individual in the non-diabetic sample.

In this thesis, a result is considered significant if the calculated probability is less than P = .01.

ACKNOWLEDGEMENTS

I would like to express my gratitude:

- to Dr. J. A. L. Gilbert, Professor of Medicine, who planted the seeds of this project and gave constant advice and encouragement as work progressed;

- to Dr. T. A. S. Boyd, Professor and Head of the Department of Ophthalmology, who took a keen interest in this work and gave much useful technical advice and helpful criticism;

- to Messrs. J. Twyman and M. Meeuse, members of the University of Alberta Hospital Department of Photography, who produced the excellent photographs found herein;

- to Dr. K. Smillie, member of the Department of Computing Science, who helped with the statistical analysis of the results.

- to the attending staff of the University of Alberta Hospital who allowed me the free use of their patients; in particular to Dr. Gordon Brown who permitted free access to his private files and allowed me to contact his patients who make up the bulk of the diabetic sample;

- to Mrs. Roberta North for her painstaking care in typing the thesis and Mr. R. McNab, medical artist, who has drawn all the figures.

This work was done during tenure of a Canadian Life Insurance Association of Canada Medical Fellowship without which this project could not have been carried out. To these people go my sincere thanks.

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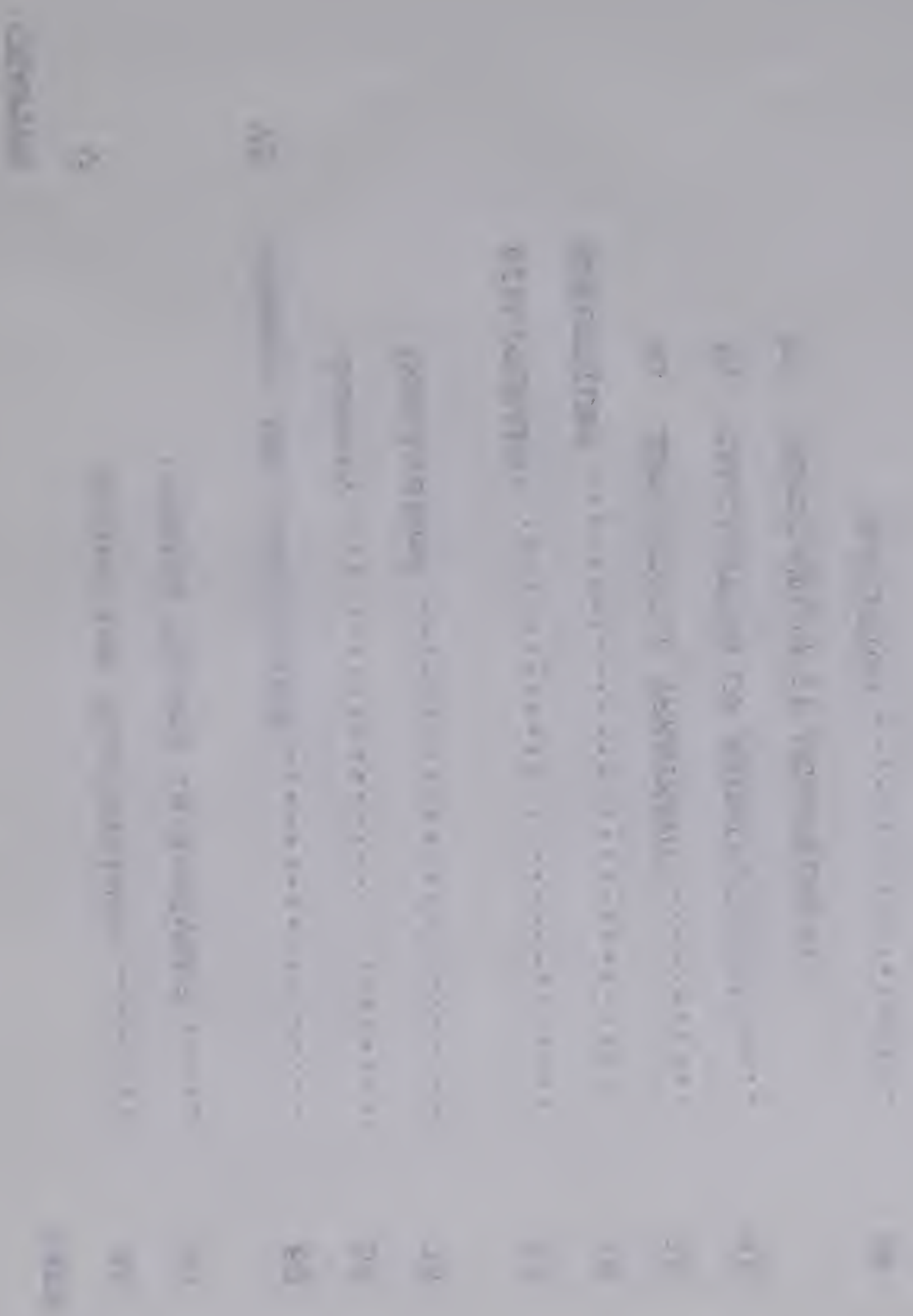

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CHAPTER I

INTRODUCTION & STATEMENT OF THE PROBLEM

INTRODUCTION:

Diabetes may be defined as "A chronic disease of varying severity, genetically determined, characterized by abnormal carbohydrate metabolism and associated with a deficiency of insulin activity, either absolute or relative, and an increased incidence of degenerative vascular disease".

The concept of diabetes as a primary microvascular syndrome has gained increasing popularity in recent years (11, 16, 46). Evidence for this concept of "capillaropathia diabetica universalis" lies in the demonstration of thickened basement membranes in almost every tissue of the body (11). Similar changes in the glomerulus, and ear-lobe have been demonstrated in prediabetics who show no abnormality in carbohydrate tolerance (18). Ditzel and his colleagues, in various publications (23, 25), claim to have found in vivo changes in the blood vessels of the conjunctiva which enable one to distinguish a diabetic from a non-diabetic. These changes consist of venular distention with alteration of the V/A ratio, capillary elongation with angularities and tortuosities, and edema of the conjunctiva. Similar changes are said to be present in significant numbers in the children of diabetic mothers (28) and in prediabetic patients (18).

If these changes are, in fact, reliable, the clinician would have a useful diagnostic tool for the detection of diabetes. Since eighty percent of the diabetic population are over 45 years (53) and since the majority of people in this age group are exposed to an ophthalmologist for correction of their presbyopia, routine photography of the conjunctival vascular bed might indeed provide a means for the detection of Diabetes Mellitus in the earliest stages.

Of the conjunctival vascular changes claimed to be present in diabetes mellitus, only two can be measured with any precision; these are the diameters of the venules and arterioles, expressed as a V/A ratio. Other changes, such as capillary elongation and tortuosity are only impressions and as such are difficult to measure with any degree of precision.

It is the purpose of this investigation to study the conjunctival microvasculature in diabetic and non-diabetic populations in an attempt to corroborate the claims of Ditzel. A representative section of the bulbar conjunctival vascular bed on the temporal side of the right eye is to be examined and photographed in triplicate in all subjects. The corresponding diameters

of paired arterioles and venules will be measured from each photograph and these measurements will be expressed as a V/A ratio.

THE PROBLEM:

The main problem involves a comparison of conjunctival V/A ratios between diabetic and non-diabetic samples to see whether any significant difference exists.

SUBSIDIARY PROBLEMS:

Subsidiary to the main purpose of this study are several other problems which will be investigated concurrently:

(1) To establish the amount of variation in venular diameter in the two samples and to compare the mean venular diameters of the two groups.

(2) To determine the amount of variation in arteriolar diameters in the two samples and to compare the mean arteriolar diameters of the two groups.

(3) To see whether the V/A ratio varies significantly between the two pairs of vessels in the same person, whether diabetic or non-diabetic.

CHAPTER II

REVIEW OF THE LITERATURE

REVIEW OF THE LITERATURE

The concept of diabetes mellitus being a microvascular disease has gained increasing popularity in recent years. Since Ballantyne's excellent monograph in 1943 (9), there have been many reviews on the subject. More recently, evidence for the presence of microangiopathy in diabetes has been demonstrated in the form of thickening of the basement membranes in the walls of capillaries in many tissues of the body, viz: ear lobe (18), ciliary process of the eye (62), glomerulus (14, 16, 21, 22, 30, 55), inner ear (41), mammary gland (44), muscle (13, 63), skin (1, 2, 51,52), peripheral nerves (33,60), conjunctiva (34), gastrointestinal tract (4) and pituitary (32).

Numerous in-vivo studies have demonstrated evidence of microvascular disease, including Megibow's studies with the plethysmograph (49) and the demonstration of increased capillary fragility by Barnes (10) and Handelsman (38).

In 1954, Ditzel and Sagild (23) published the first of several reports regarding their studies of the conjunctival microvasculature. In a further report (24) two years later, they divided changes in the conjunctiva into two groups: (See Table I)

A. Those due to aging but accelerated by the diabetic process.

B. Those independent of age and specific for the diabetic process.

TABLE I

CHANGES IN THE CONJUNCTIVAL VASCULAR BED IN DIABETES MELLITUS (According to Ditzel (24)).

A. CHANGES DUE TO AGING BUT SPEEDED UP BY THE DIABETIC PROCESS

(a) Vascular:

i. Arteriolar wall irregularities
ii. Venular wall irregularities
iii. Venular sacculations

(b) Perivascular:

i. Conjunctival haemorrhages
ii. "Hyaline" infiltration

B. CHANGES INDEPENDENT OF AGE AND SPECIFIC FOR THE DIABETIC PROCESS:

(a) Vascular:

i. Altered V/A ratio
- Venular dilatation
- Mild arteriolar constriction

ii. Capillary elongation
- Hairpins
- Corscrews
- Angularities

(b) Perivascular:

i. Edema of the conjunctiva

The only vascular changes in the conjunctiva which can be measured precisely are venular and arteriolar diameters expressed as a V/A ratio. There is a considerable difference in the range of reported values for the V/A ratio in normal subjects. Ditzel (23) claimed that all normal V/A ratios lie between 3:1 - 2:1. Bech (12), found ranges of 4.5:1 - 1:1 in his 24 normal controls. Meighan (50), in sixty normal subjects, found ranges of 4:1 - 2:1. Landau & Davis (43) found a fairly constant ratio of 3:2 in normal subjects which increased to between 4:1 - 6:1 in the presence of arteriosclerosis.

Bech (12) found a variation in the V/A ratio of different pairs of vessels in the same person. This variation was greater in normals than in diabetics.

No correlation between age and V/A ratio was noted by Bech (12) or Landau (43). Ditzel (24), however found smaller V/A ratios in older diabetics and normals.

Both Ditzel (25) and Bech (12) found no relation between V/A ratio and duration of diabetes. No relationship has been found (26) between V/A ratio and blood sugar levels or insulin dose.

Ditzel (26) found a diurnal variation present in venules. The maximal dilatation was in the forenoon

while minimal dilatation was seen in late afternoon. Although this may roughly correspond to the diurnal fluctuations of glucocorticoids, no relationship between infused ACTH and venular dilatation was found (26).

Ditzel (28) demonstrated conjunctival vascular changes in the children of diabetic mothers. These consisted of capillary elongation with angularities and tortuosities in 48% of the children compared to 4% of controls. 43% of the offspring of diabetic mothers had abnormal V/A ratios above 3:1; the V/A ratio in controls was always less than 3:1.

Camerini-Devalos et al. (18), in an extensive study of prediabetics, found 92% had a V/A ratio significantly larger than the mean of 2.29 (∓0.26) found in the control group.

The presence or absence of microaneurysms outside the retina in diabetes mellitus has been a subject of much controversy. Ashton (6) made flat preparation of twenty-one post-mortem conjunctivae and failed to find evidence of microaneurysm formation. Ditzel (23) found microaneurysms in an equal proportion (4%) of both diabetic and non-diabetic samples. Friedenwald (31) found microaneurysms in 5% of diabetic conjunctivae which he considered to be higher than in the non-diabetic population.

McCulloch and Pashby (48) found saccular microaneurysms in 55% of diabetics and 14% of non-diabetics. These findings were later supported by Weinstein and Forgacs (58). Funahasi and Fink (34) found microscopic evidence of saccular microaneurysms in 63.6% of diabetics undergoing cataract surgery. All the microaneurysms, however, were found in the exposed portion of the bulbar conjunctiva. This emphasized Cook's thesis that findings in the conjunctiva should be interpreted with some reservation since this part of the eye is constantly exposed to trauma and inflammation (20). Cook examined 250 diabetics and 250 controls of comparable age and found only a slightly increased incidence of microaneurysms in the diabetics which he attributed to concomitant hypertension. In short, he was unable to substantiate the claims that specific vascular aneurysms, analagous to those in the retina, occur in the conjunctivae of the diabetic subject.

CHAPTER III

THE ANATOMY AND BLOOD SUPPLY OF THE CONJUNCTIVA

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The conjunctiva is a thin, transparent mucous membrane which derives its name from the fact that it attaches the eyelids to the eyeball (59). It lines the posterior surface of the eyelids and is then reflected on to the globe where it becomes known as the BULBAR CONJUNCTIVA. The bulbar conjunctiva is so thin and transparent that the white sclera shows through, giving rise to the "white of the eye." This membrane lies loosely on the underlying tissues. Up to a point about three mm. from the cornea it is separated from underlying Tenon's capsule by loose areolar tissue, the substantia propria, in which lie the conjunctival vessels. Between Tenon's capsule and the sclera is loose episcleral tissue in which lie the anterior ciliary arteries which form the pericorneal plexus.

(a) Structure of the Conjunctiva:

Duke-Elder (29) divides the conjunctiva into two layers:

i. Epithelial layer

ii. Substantia propria — Adenoid layer / Fibrous layer

i. The epithelial layer varies in structure with the location. The tarsal areas have two to four layers of polygonal and cuboidal cells. The bulbar conjunctiva is thicker, resembling true stratified epithelium, but it never becomes keratinized. The basal cells may contain

pigment granules in this region (59), particularly near the limbus. Goblet cells are found interposed between the epithelial cells especially in the fornices.

ii. The substantia propria consists of two portions - a superficial adenoid layer and a deeper fibrous layer. The adenoid layer consists of a fine reticular mesh which contains lymphocytes. The deep fibrous layer of the substantia consists of collagen and elastic tissue. In it are the conjunctival vessels and nerves supplying the mucous membrane.

(b) Conjunctival Vessels: (FIG 1)

1. - Arteries:

The arterial supply to the conjunctiva comes from three sources (59):

- Superior tarsal arcade
- Inferior tarsal arcade
- Anterior ciliary arteries

Of these the superior tarsal arcade, as far as the upper eyelid is concerned, supplies the greatest area.

(a) Superior Tarsal Arcade:

The superior tarsal arcade of the upper lid is located between the two layers of the levator palpebrae superiores at the upper end of the tarsal plate. Perforating branches penetrate Muller's muscle to reach the conjunctiva where ascending and descending branches are given off. The ascending branches pass upwards towards the fornix, bend

around and enter under the bulbar conjunctiva as the posterior conjunctival arteries, which supply the entire bulbar conjunctiva save for an area four mm. wide around the limbus, where they anastomose with the anterior conjunctival arteries which arises from the anterior ciliary artery. The descending branches of the penetrating vessel supply the majority of the tarsal conjunctiva. The superior tarsal arcade of the lower lid is a very inconstant structure but when present its location and course is similar to that described for the upper lid.

(b) Inferior Tarsal Arcade:

The inferior tarsal arcade is the larger of the two and is located in front of the tarsal plate, three mm. from the free edge of the eyelid. Perforating branches pierce the lid to reach the conjunctiva at the subtarsal fold. These perforating branches divide into tarsal and marginal twigs. The tarsal twigs anastomose with the descending portions of the perforating vessels from the superior tarsal arcade.

(c) Anterior Ciliary Arteries:

The anterior ciliary arteries arise from arteries supplying extraocular muscles. Each muscular artery contributes two branches except that to the lateral rectus which only contributes one. These branches lie in the episcleral tissue and four mm. from the limbus they penetrate the sclera to serve deeper structures. At

this point they give off the anterior conjunctival arteries which pass forward in the episcleral tissue and anastomose with each other, forming a series of arcades parallel to the corneal margin. Anteriorly they form the pericorneal plexus. They send twigs back posteriorly which anastomose with the posterior conjunctival vessels.

2. - Veins:

Veins accompany the corresponding artery but are larger and more numerous. Their drainage is inconstant but for the most part they drain into the palpebral veins.

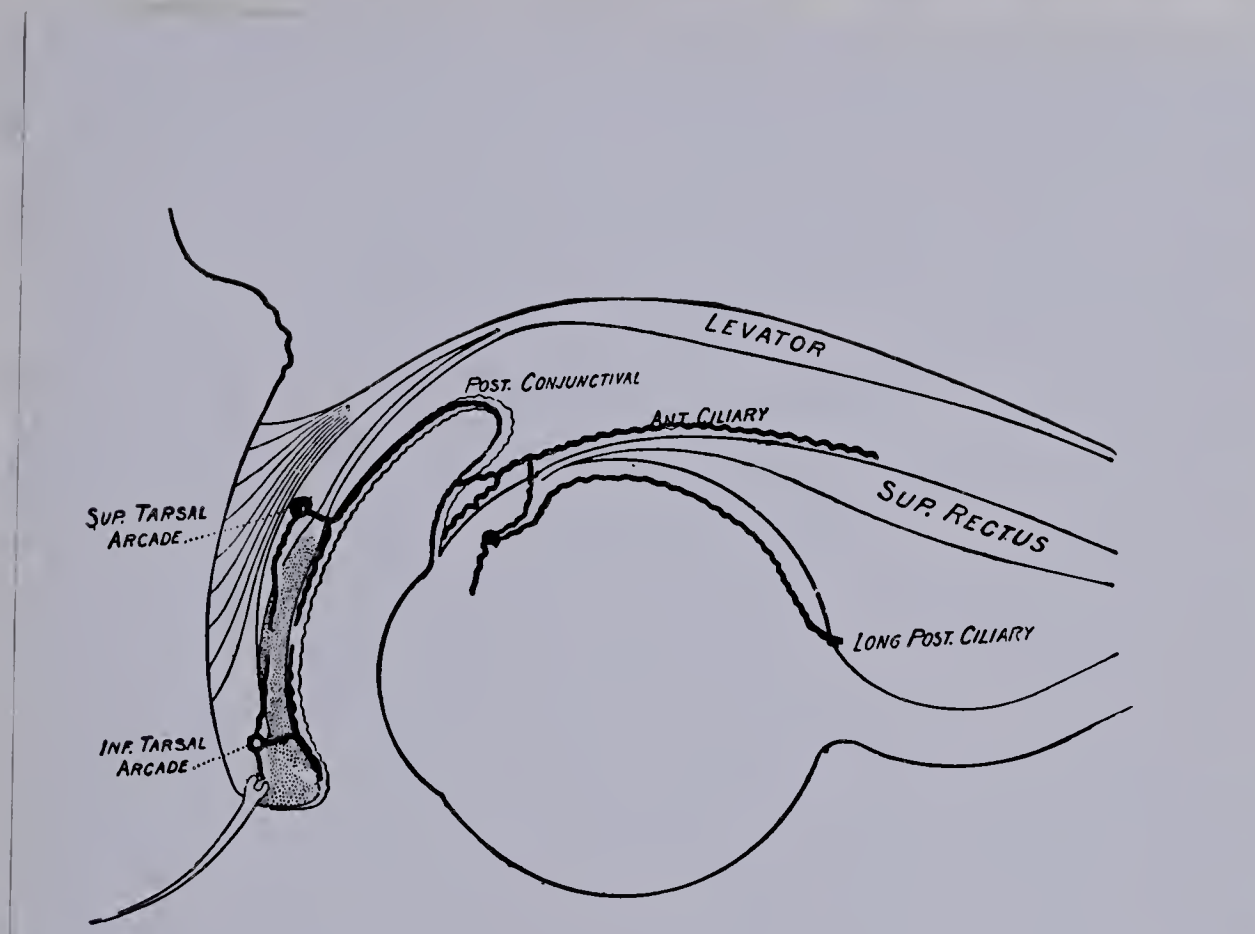


FIGURE I

BLOOD SUPPLY OF THE BULBAR CONJUNCTIVA

(c) The Biomicroscopic Appearance of Conjunctival Vessels

The walls of the blood vessels are indistinguishable from the sclera and are difficult to discern. What is usually studied is a red column of blood which changes in diameter as it moves from one vessel to another (15).

The tissue of the bulbar conjunctiva is wet and glistening in health and the vessels are easily seen. However in the presence of edema the arterioles, which lie slighly deeper than the venules, become indistinct (15). For accurate analysis of the conjunctival micro-vasculature, the various type of blood vessels which exist there must be correctly identified. Five types of vessels can be identified: (FIG.II)

i. Arterioles:

By definition, an arteriole is a small artery with diameter less than one hundred microns. The vessels are filled with red, watery blood which flows more rapidly than in the corresponding venule. The direction of flow is always toward progressively smaller vessels. The calibre of arterioles is even and regular. Their course is relatively straight.

ii. Venules:

The corresponding venule is generally larger, containing darker red, slower moving blood, which flows progressively toward vessels of larger calibre. The course, in comparison to arterioles, is more sinuous and the calibre is far from regular with focal areas of dilatation common.

The ratio between venule and arteriole is variable. The range of normals varies with the author. Ditzel (24) claims all his normal ratios were between 2:1 - 3:1; Bech et al (12) found ranges of 1:1 - 4:5:1 in 24 non-diabetics. Bloch (15) says 1.5:1 - 2:1 is the range of normal. Spencer Meighan (50), in sixty normal subjects, reported the following proportion of ratios:

2:1 - 10%

3:1 - 66.7%

4:1 - 23.3%

Landau and Davis (43) found a constant ratio of 3:2.

iii. Capillaries:

Capillaries are fine hair-like structures which vary in diameter, admitting 1-6 r.b.c. (54). These vessels arise from metarterioles and terminal arterioles, form a fine network throughout the conjunctiva, then join metarterioles and venules distally. Like

capillaries elsewhere, many of them are collapsed and invisible at any given time (35).

iv. Metarterioles:

Metarterioles arise from terminal arterioles, course through the conjunctiva giving off capillaries in their proximal portions and receiving them in their distal segments. They then join with similar vessels to form venules. They are recognized as being larger than capillaries and the blood flow within is faster than in adjacent capillaries.

v. Aqueous Veins:

Aqueous veins, numbering 20-30, represent exit channels for aqueous humour which connect Schlemm's Canal with the conjunctival and episcleral venules (5). They emerge from deep ocular structures 2 mm from the limbus and are seen maximally between one and five and seven and eleven o'clock positions in relation to the cornea. These vessels contain clear fluid or fluid mixed with blood, separated in different strata, thus retaining their separate identities.

(d) The Structural Unit of the Capillary Bed: (FIG.III)

Using the mesentery of the dog and the mesoappendix of the rat, Chambers and Zweifach (19) demonstrated a

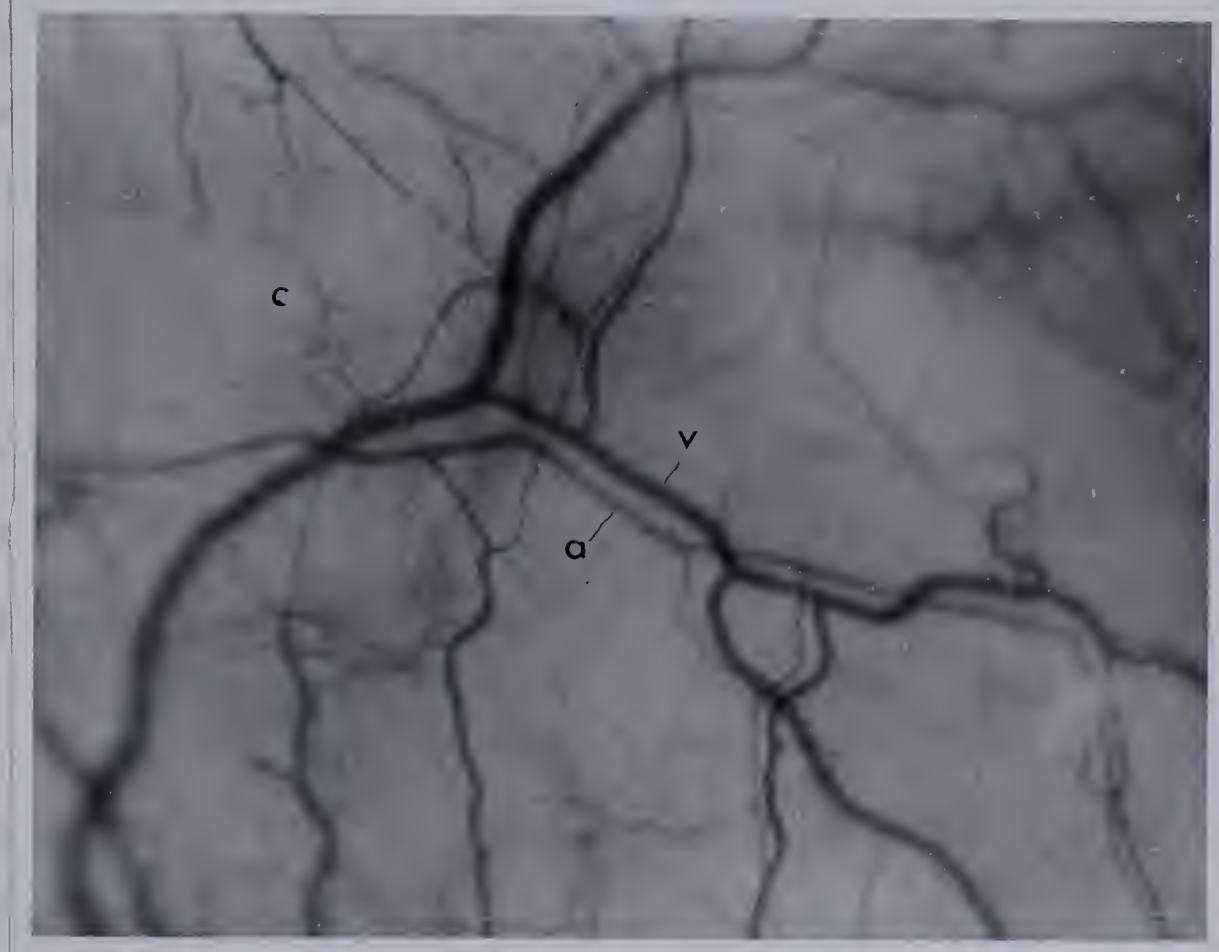


FIGURE II

TYPICAL CONJUNCTIVAL VASCULAR BED

(a: arteriole; v: venule; c: capillaries)

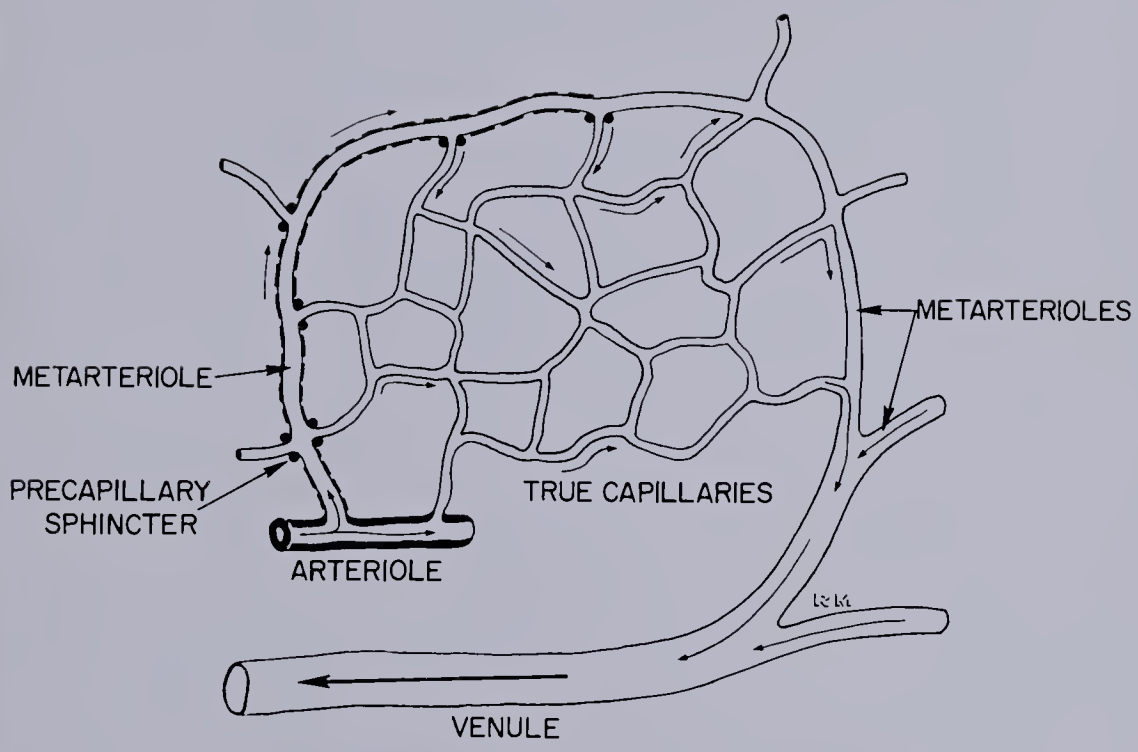
STRUCTURAL UNIT in the architecture of the capillary bed. They observed certain vessels, of capillary dimension, through which the blood was flowing more rapidly than through the capillaries, adjacent to them. These vessels, which they named metarterioles, originate from terminal arterioles, course through the vascular bed only to merge with similar channels to form venules. Along the proximal portions of metarterioles, capillaries branch off and carry blood away. Along the distal portion the capillaries drain blood back. Encircling the junction of capillaries in the proximal portion of the metarterioles are smooth muscle fibres, the pre-capillary sphincters, which are under neuronal and humoral control. Capillaries also branch off terminal arterioles directly and also have a pre-capillary sphincter.

This concept of a structural unit was not accepted by all. Webb & Nicol (57) were unable to distinguish thoroughfare channels in the wing of the bat; Lutz & Fulton (47) observed vein-to-vein anastomoses and arteriole-to-arteriole anastomoses but were unable to distinguish A/V communications as Chambers and Zweifach did.

Regarding the conjunctival vascular bed, Lee & Holze (45) interpreted their findings in the conjunctival vessels as generally being in accord with the concepts

of Chambers & Zweifach. Kunitomo (42) in his studies of the conjunctival microvasculature, does not mention A/V anastomoses. Grafflin & Bagley (36) and Grafflin & Corddry (37) stated they found endless, random conjunctival vascular patterns which did not resemble the structural unit described above. Meighan (50) described vascular patterns in the bulbar conjunctiva of man which resembled, to a limited degree, that described by Zweifach in the mesentery of animals.

In 1954 Zweifach (64) modified his earlier statements indicating that the structural unit he described in the animal mesentery was present in other tissues but in a modified form.



STRUCTURAL UNIT OF THE CAPILLARY
BED ACCORDING TO ZWEIFACH

FIGURE III

THE STRUCTURAL UNIT OF THE CAPILLARY BED

CHAPTER IV

METHODS AND PROCEDURES

SELECTION OF SUBJECTS:

Two populations of patients were sampled: a Diabetic and a non-Diabetic (control) population.

(a) Diabetic Sample:

57 Diabetic patients were used for this study. The majority of these were taken from the practice of Dr. G. Brown. The subjects were selected if they fulfilled all the criteria set out in Table II (page 22).

(b) Control sample:

66 control subjects were used. They fulfilled a rigid set of criteria as put forth in Table III (page 23). Most of these patients were taken from the Psychiatric wards and were all free from any organic disease.

SCREENING TEST FOR DIABETICS AMONGST THE CONTROL GROUP:

The presence of Latent Diabetes in the control sample was ruled out using the two-hour postprandial blood sugar determination recommended by the American Diabetes Association. Every potential control subject was given a control meal containing 200 grams of carbohydrate (Table IV, page 24). Two hours later a blood sugar determination was made using the Auto-Analyzer ferricyanide

TABLE II

CRITERIA FOR ACCEPTANCE AS A DIABETIC:

1. Between the ages of 15 and 45 years.

2. Diagnosed as diabetic on the basis of abnormal Glucose Tolerance Tests and repeated glycosuria.

3. Taking insulin parenterally.

4. Normotensive (Blood Pressure below 150/100)

5. No other concomitant eye disease (other than retinopathy)

6. No allergies (hayfever, etc.)

7. No systemic disease which has microvascular manifestations.

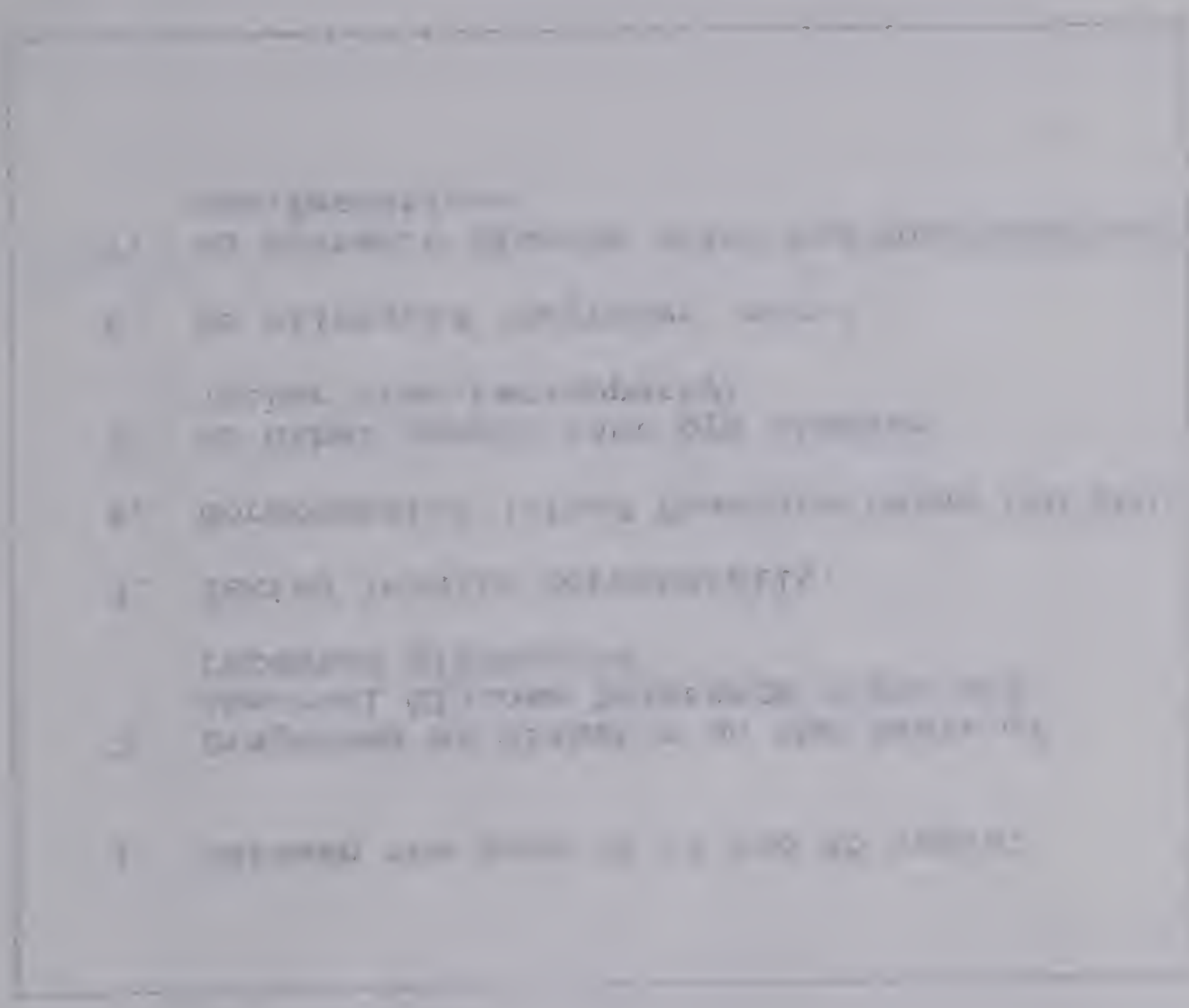


TABLE III

CRITERIA FOR ACCEPTANCE AS A NORMAL (Control)

1. Between the ages of 15 and 45 years.

2. No family history of diabetes.

3. A two-hour blood sugar, after a standard meal containing 200 gm. of carbohydrate (Table II) was below 110 mgm % on the Auto-Analyzer.

4. Normotensive (Blood Pressure below 150/100)

5. No eye disease present.

6. No allergies (hayfever etc.)

7. No systemic disease which has microvascular manifestations.

8. No signs or symptoms of Pre-Diabetes:

   i Repeated miscarriages

   ii Babies over ten pounds

   iii Toxaemia

   iv Hydramnios

   v Symptoms hypoglycemia

   vi Repeated premature babies

   vii Repeated stillbirths

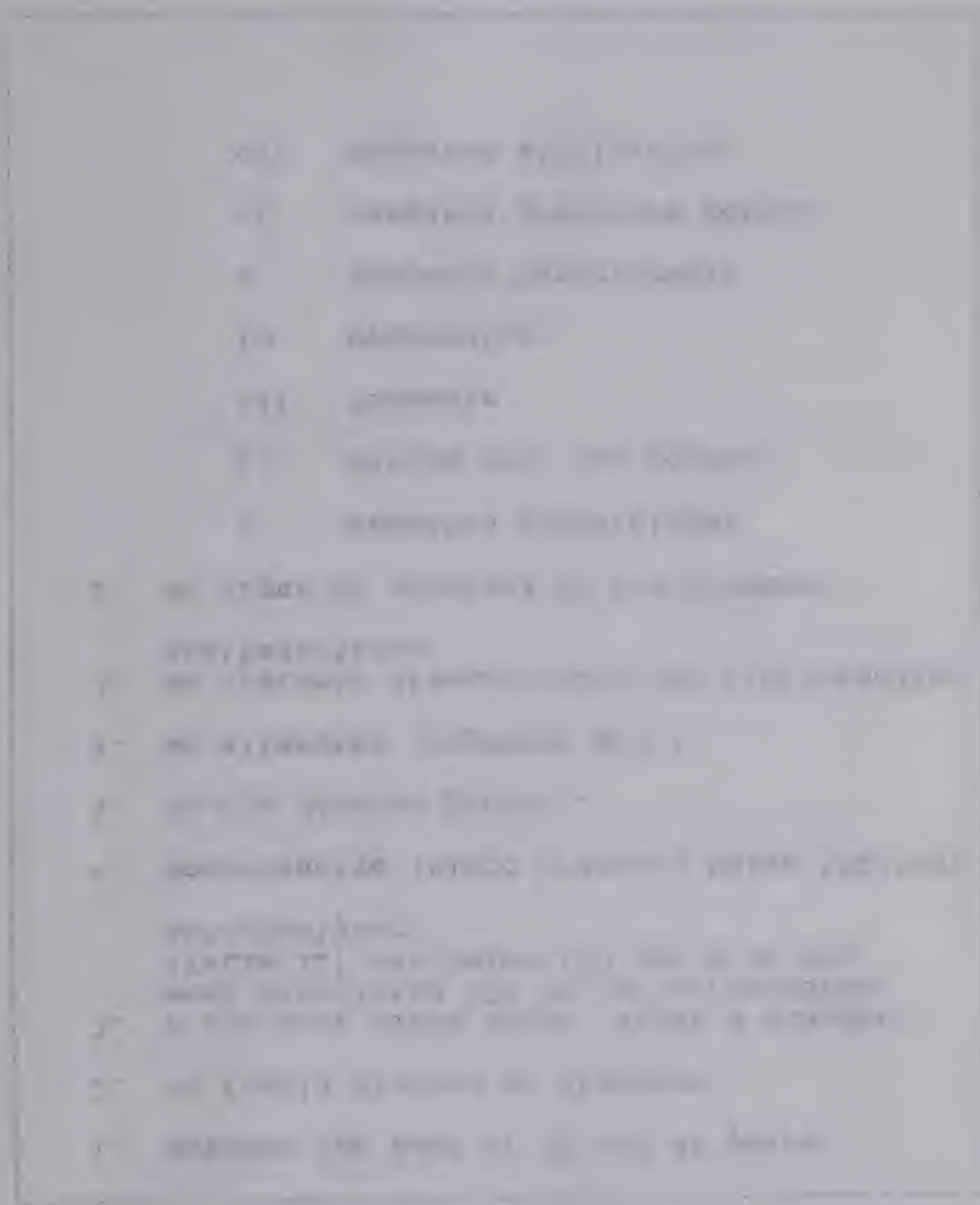


TABLE IV

STANDARD TEST BREAKFAST CONTAINING 200 GRAMS OF CARBOHYDRATE

	Carbohydrate Content
1 Cup <u>sweetened</u> Fruit Juice	60 gms.
1 Cup Cooked Cereal with two Tablespoons sugar (15 gms each)	60 gms.
3 Slices toast with liberal amounts of jam.	60 gms.
1 cup milk	12 gms.
1 cup coffee with 2 lumps sugar	10 gms.
	200 gms.

method. Only those people with a blood sugar reading below 110 mgm % were included in the control group. Only a very few persons with a normal two hour post-prandial sugar determined in this manner will have a diabetic glucose tolerance curve.

THE EQUIPMENT:

A Bausch & Lomb Universal Slit Lamp was used for all examinations of the bulbar conjunctiva.

(a) The Microscope (FIG IV, page 29)

Essentially, the microscope is a binocular type fitted with a revolving nosepiece which carries two paired objectives. All the observations were done using a 10x ocular in conjunction with an objective of focal length 40.0 mm. There are two knobs for adjusting the instrument: Knob (A) allows the microscope to be racked forward and back: knob (B) allows for a range of up and down movement.

(b) The Instrument Table (FIG IV, page 29)

The table which supports the microscope may be raised and lowered using the hand wheel (c) and can be locked at any height by the knob (D).

(c) Head and Chin Rest (FIG V, page 30)

The head and chin rest (G) allows for immobilization of the subject's head. The apparatus has three adjustments: the chin can be raised or lowered by the knurled ring (H) in order that the head rest will conform to the length of face of various persons.

The head rest can be tilted by means of the screw (I). The entire head and chin rest can be rotated about a vertical axis by loosening a set-screw (J).

(d) The Illuminating System (FIG.V, page 30)
The light source for the illuminating system comes from a 19 volt 2½ amp. lamp (K), having a straight heavy filament. A diffusing lens is used to break up the filament structure in the image. The beam of light is passed through a slit and an image is formed by the illuminating lens (L). The resultant beam consists of COLD light which cannot be felt by the subject and provides adequate illumination for focussing the microscope.

(e) The Camera (FIG. IV, page 29)
An Asahi Pentax, single-lens reflex camera (E) was used for photographing the conjunctival vessels. It was attached to one of the collimator tubes of the microscope using the Asahi microscope attachment. The ocular was left in place. The camera was synchronized with the electronic flash unit using a shutter speed of 1/50 sec. The camera was fired using a cable release (F) which minimized movement of the camera

as the picture was taken.

(f) The filter:

A Kodak Wratten Gelatin filter #82-A was placed in front of the objective lens of the microscope in order to filter out the red, yellow and green light waves thus rendering the panchromatic film relatively orthochromatic. A very light filter was chosen because one could not afford to have too much light absorbed as already a good deal was lost while passing through the prisms of the microscope. Since such a pale blue filter was used, only a portion of the red waves were effectively filtered out (FIG.VI, page 33).

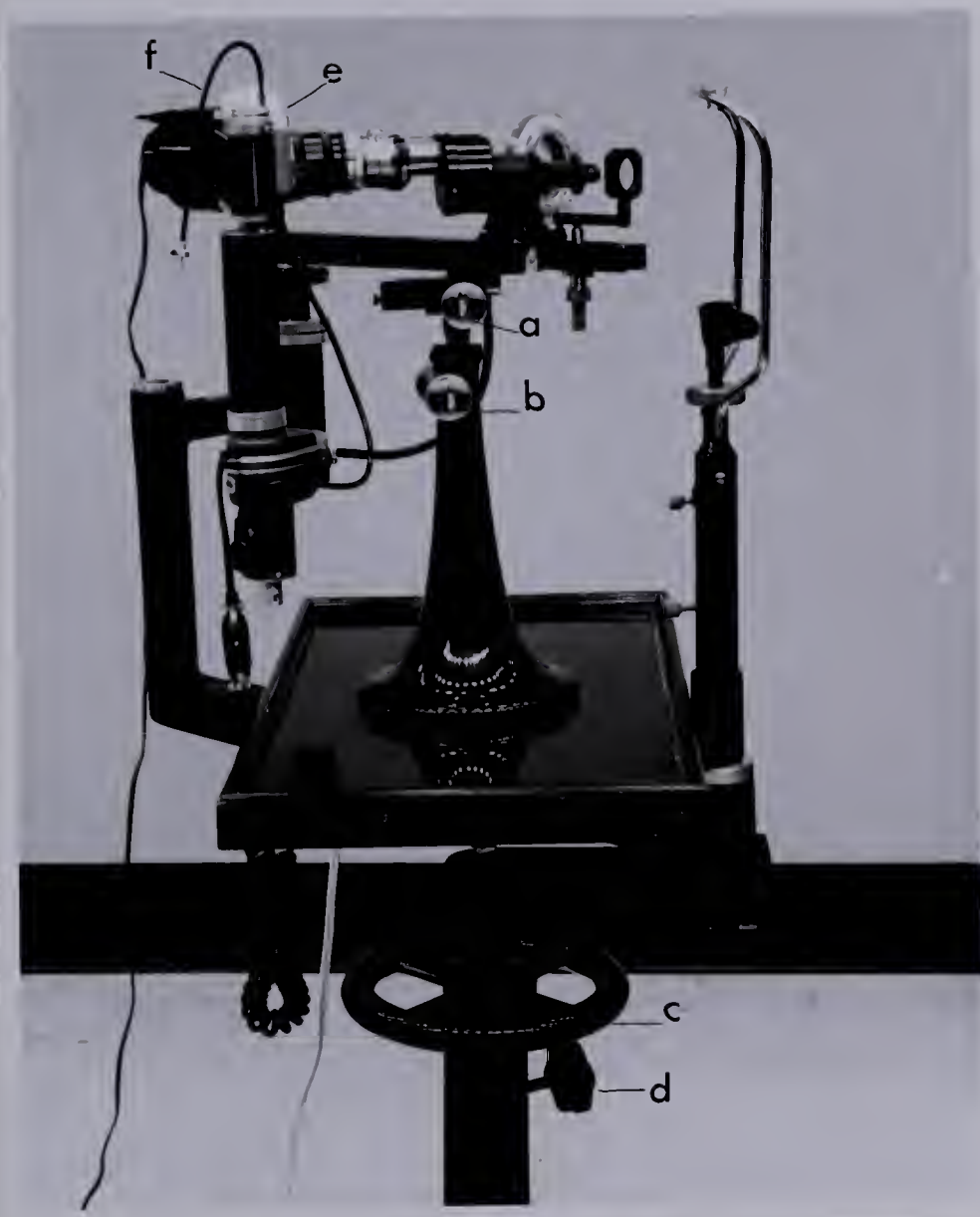


FIGURE IV

BAUSCH & LOMB UNIVERSAL SLIT LAMP

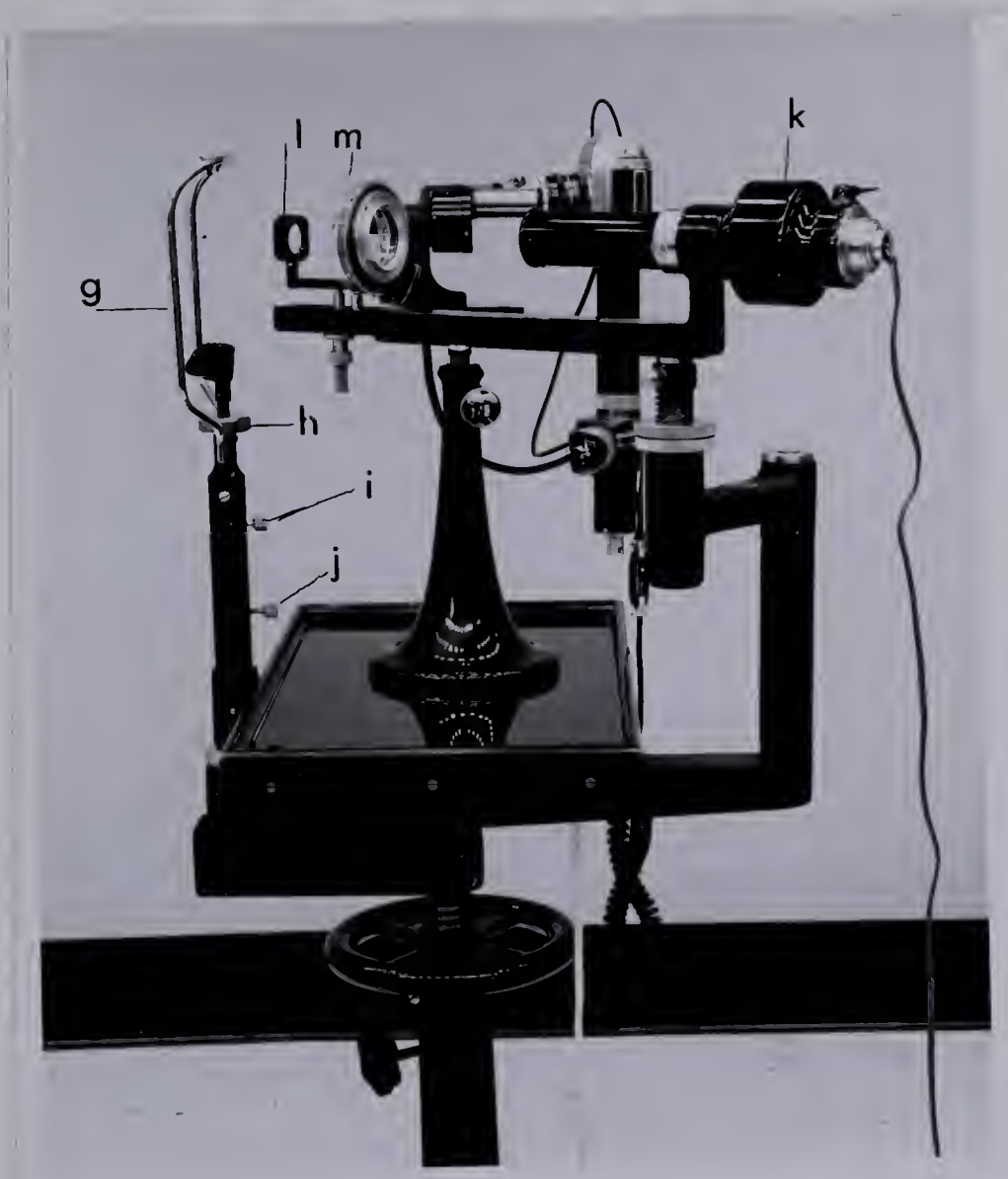


FIGURE V

BAUSCH & LOMB UNIVERSAL SLIT LAMP

(g) The film:

The type of film used was Ansco Super-Hypan which was arrived at solely by trial and error. The film has the following characteristics:

- Contrast:	Gamma values ranging from 0.7 - 0.8.
- Grain:	Medium; considerably finer than that associated with traditional high-speed films.
- Speed	- 500/7°
- Resolving Power	80-90. lines/mm

Spectral sensitivity:

A wedge spectrogram shows that this film is panchromatic, being sensitive to light waves between 4000Å - 6400Å (1 Å = 1-10 millionth mm). Ideally spectral sensitivity should be defined with reference to a spectrum which contains equal amounts of energy in each colour band. Since the spectral quality of our flash unit contains different amounts of energy per unit wave length; the wedge spectrogram should be interpreted in light of this (FIG. VI, page 33).

(h) The Flash Unit (FIG. V, page 30)

A Speed-Light Close-up ring (M) of diameter 3½" was used as a light source for exposing the film. It was located 6 - 8" from the conjunctiva, and was of the following characteristics:

Light output = 30 watt-seconds

Colour temp = 5800 degrees Kelvin

Flash duration 1/1000th of a second

Firing Voltage = 450 volts

The condensor for firing the Ring strobe is fixed to the base of the camera.

Development

The film was developed in "Hyfinol" a commercial developing solution. The film was over-developed in solution at 65° F for a period of nine minutes (Normal = 6 minutes) to give maximum contrast.

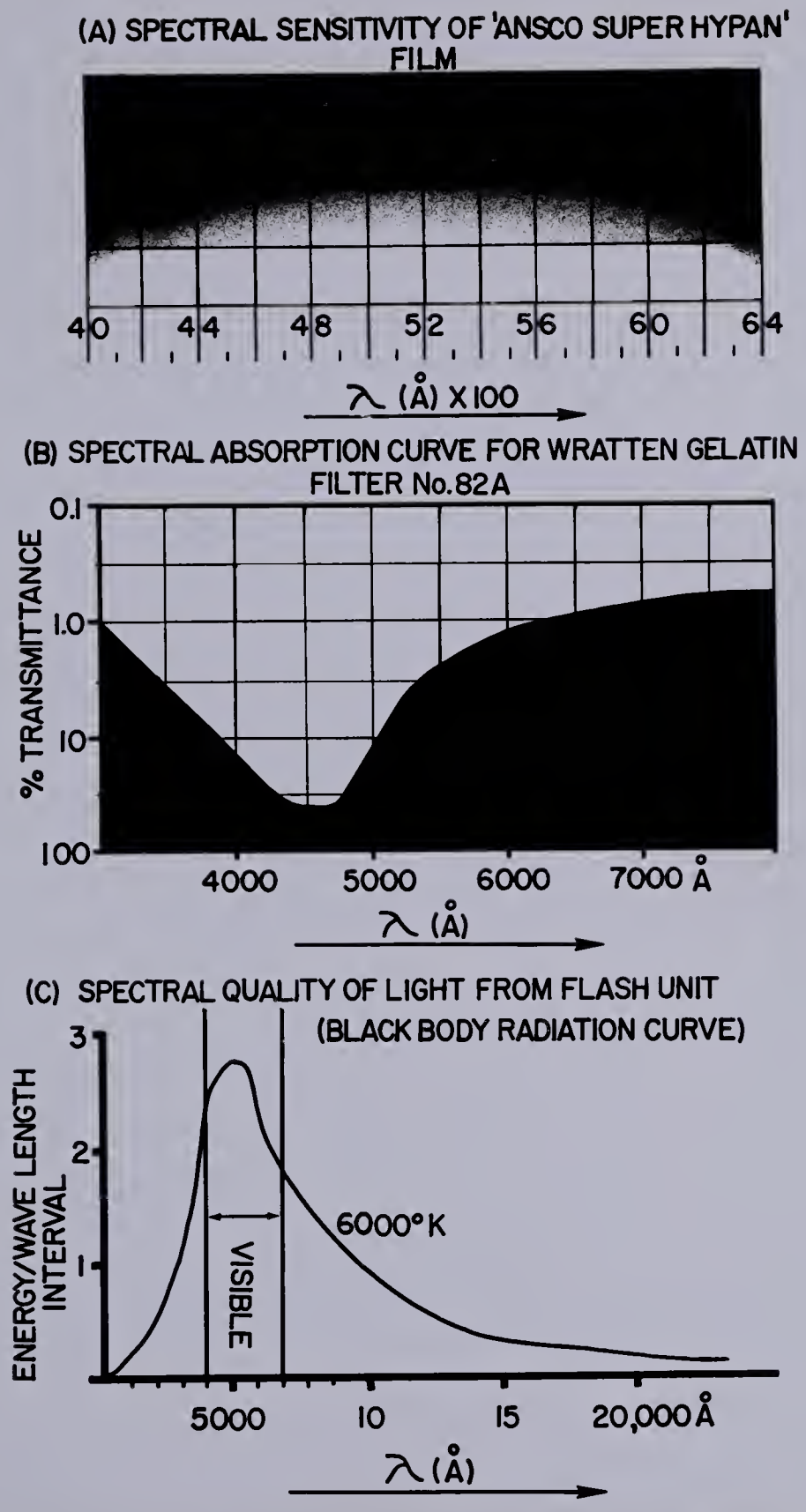


FIGURE VI

COMPARISON OF SPECTRAL SENSITIVITY OF FILM, SPECTRAL ABSORPTION CURVE OF FILTER AND SPECTRAL QUALITY OF LIGHT FROM FLASH

PROCEDURE:

The bulbar conjunctiva was photographed with the subject seated at the instrument table. The table's height was adjusted to suit the patient and his head was then placed in the head and chin rest. The eyes were then focussed on a point off to the patient's left, thus exposing the temporal portion of the bulbar conjunctiva of the right eye (T) (FIG. VII, page 35).

A portion of the conjunctiva, midway between the limbus and outer canthus was selected and was brought into focus using the free collimator tube of the microscope. The arm carrying the illuminating light was maneuvered to provide maximum illumination of the conjunctiva and facilitate focussing of the microscope. A portion of the vascular bed containing at least ONE pair of vessels (an arteriole and venule) was located at random and three pictures were then taken of this selected area.

All photography was performed between 10:00 and 12:00 in the morning since this is the period of supposed maximal venular dilatation (26).

MEASUREMENT OF VESSELS FROM THE PHOTOGRAPHS:

The negative images of the conjunctival vascular beds were selected at random from a box and projected on

FIGURE VII

SUBJECT LOOKING OFF TO HER LEFT, THUS EXPOSING THE TEMPORAL PORTION OF THE RIGHT BULBAR CONJUNCTIVA (T).

to a matte white screen using a Leitz 35 mm projector with a 1:2.5/120 mm lens. The screen was located at a fixed distance of five feet from the slide (FIG.VIII, page 37). Projection magnified each negative ten times (See Appendix B). Calibre of the vessels was measured using a pair of calipers, a fine calibrated rule and a magnifying glass (FIG.IX, page 38).

After measurements had been taken with the calipers, they were applied to a fine calibrated scale and readings, in arbitrary units, were taken with the aid of a magnifying glass to ensure the most accurate readings possible (FIG. X, page 39).

TEN measurements were made of the diameters of the arterioles and venules of each vessel pair. Where two pairs of vessels were visible in one picture, ten measurements were made of EACH pair.

Each diameter measurement was used to obtain the approximate cross-sectional area of the vessel. (Formula = $\pi\ d^2/4$). The ten cross-sectional areas for each arteriole were then summated. Since π and 4 are constants for every calculation they may be ignored, and the diameter alone is then squared for each measurement.

The mean cross-sectional area of each arteriole and venule is thus obtained from the ten measurements

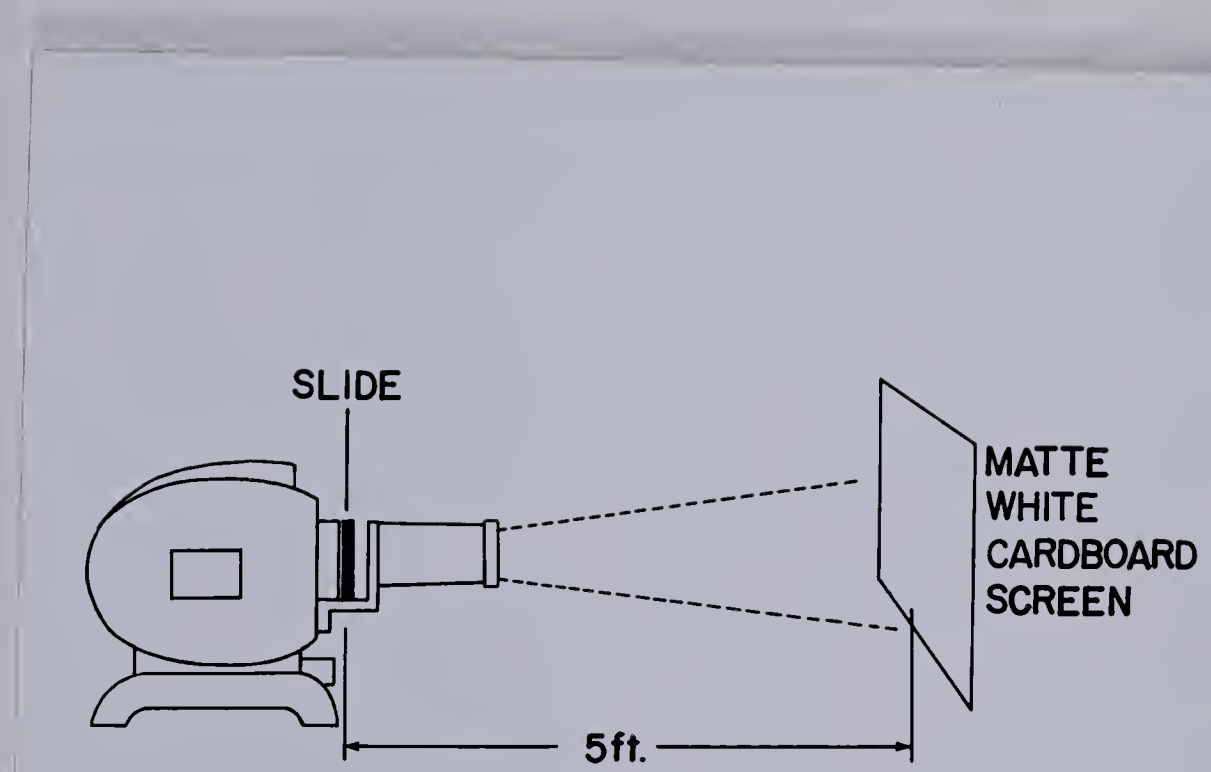


FIGURE VIII

FIGURE SHOWING PROJECTOR WITH SLIDE LOCATED FIVE FEET FROM A MATTE WHITE SCREEN

FIGURE IX

FIGURE SHOWING CALIBRATED RULE (A), MAGNIFYING GLASS (B) AND CALIPERS (C) USED IN MEASURING THE VESSELS

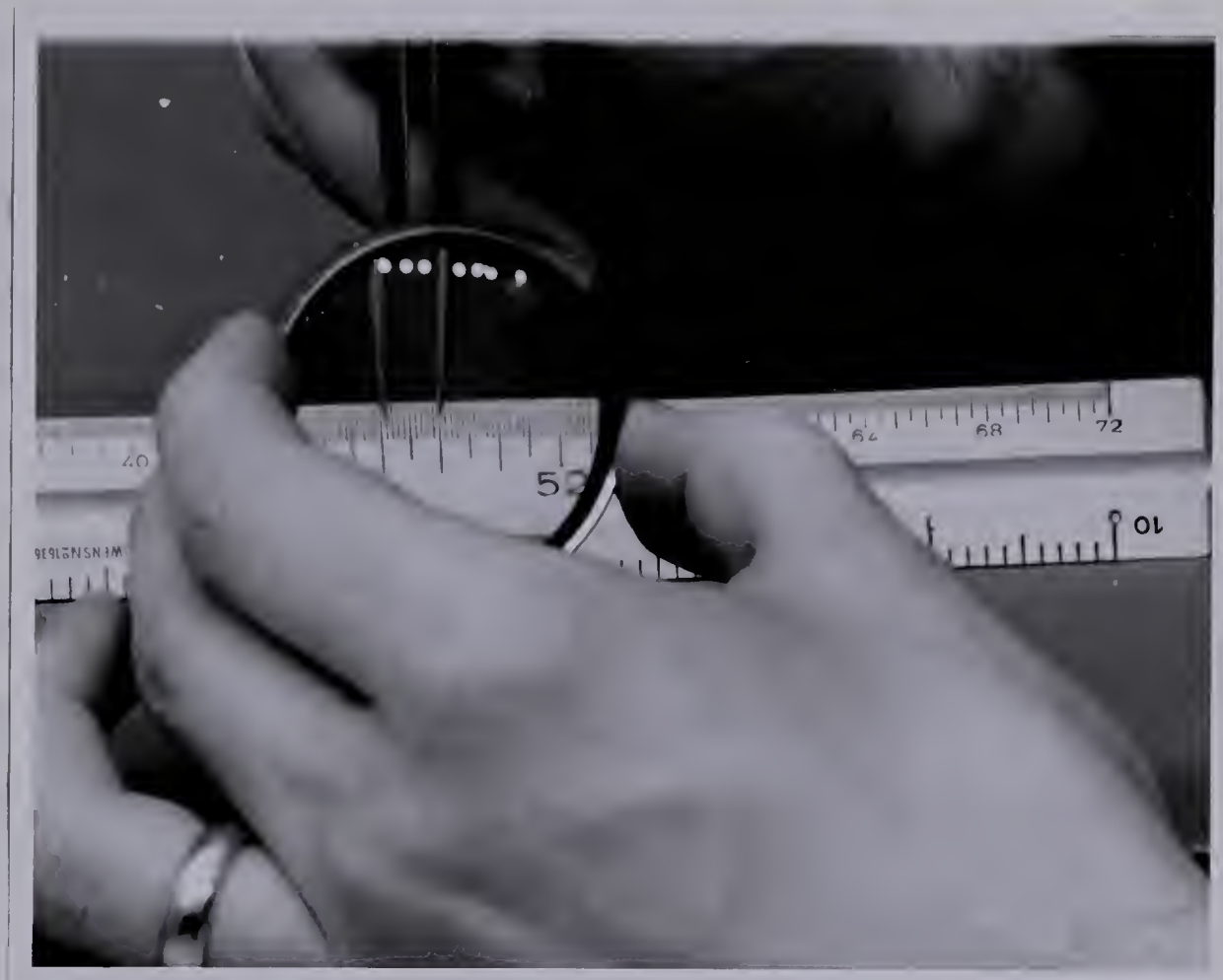


FIGURE X

FIGURE SHOWING TECHNIQUE OR TAKING READING OF THE CALIPERS USING CALIBRATED SCALE AND MAGNIFYING GLASS

and these are the figures which are included in the tables from which the results are derived. The mean cross-sectional area of each venule is divided by the mean cross-sectional area of the corresponding arteriole and the resultant V/A ratio is expressed as a decimal fraction.

The calibre values were converted into total cross-sectional area because the latter expresses "vascular capacity" more accurately than does the sum of calibres (i.e. diameters). The following example (FIG.XI, page 41) shows the fallacy of non-conversion. Four measurements made of two vessels, show the following diameters: 10, 10, 12, 12 and 10, 10, 8, 16 units. If the sum of the diameters is taken to represent vascular capacity, then the circulations are equal. However if the cross-sectional areas of the vessels are the criteria of capacity, then converting the diameters into the corresponding cross-sectional area reveals that the second vessel has the larger vascular capacity.

STATISTICAL TREATMENT:

The following variables were recorded in both the diabetic and non-diabetic samples for each arteriole-venule pair:

(a) Mean arteriolar cross-sectional area.

(b) Mean venular cross-sectional area.

(c) V/A ratio in terms of a decimal fraction for each pair of vessels.

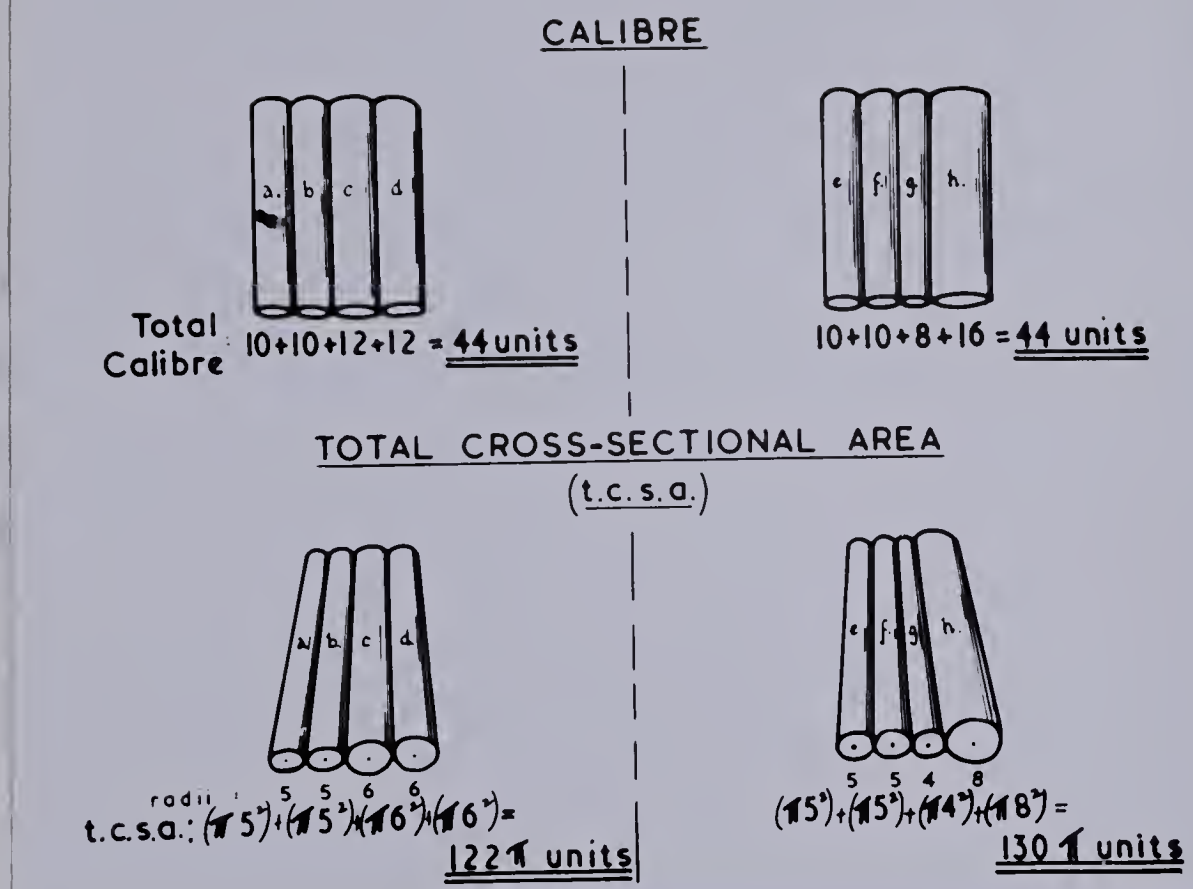


FIGURE XI

ILLUSTRATION SHOWING THE FALLACY OF NON-CONVERSION OF DIAMETER MEASUREMENTS TO MEAN CROSS-SECTIONAL AREAS, WHEN ESTIMATING VASCULAR CAPACITY

The mean arteriolar cross-sectional areas, venular cross-sectional areas and V/A ratios of diabetic and non-diabetic samples were plotted on frequency-distribution curves. Three comparisons were made:

(1) Between the V/A ratios of diabetics and non-diabetics.

(2) Between the mean venular cross-sectional areas of diabetics and non-diabetics.

(3) Between the mean arteriolar cross-sectional areas of diabetics and non-diabetics.

Intra-individual variation of V/A ratios in the diabetic and non-diabetic samples was calculated when more than one V/A pair was present in the photograph. This test of variation was performed using the Wicoxon Test for the Paired Case (3).

CHAPTER V

RESULTS & DISCUSSION

RESULTS:

Studies were carried out on 57 diabetics and 66 non-diabetics (controls). All members of both groups were between the ages of 15 and 45. The frequency-distribution of age groups is set out in two histograms (FIG. XII, page 45).

The lower of the two histograms illustrates the range of ages of the non-diabetic (control) subjects. The upper shows a similar range of ages for the diabetic sample.

In this section the data will be presented under the following headings:

I -Total cross-sectional area of the arterioles.

II -Total cross-sectional area of the venules.

III -V/A ratios in terms of decimal fractions.

IV -Intra-individual variations of V/A ratio.

I- Total cross-sectional area of the arterioles:

Figure XIII (page 46) contains the data on mean cross-sectional areas of conjunctival arterioles for the 66 non-diabetics. Figure XIV (page 47) contains the data on mean cross-sectional areas for the 57 diabetics.

It should be noted that there is a marked skew

to the right for both of these frequency distribution curves. A correction will be applied later.

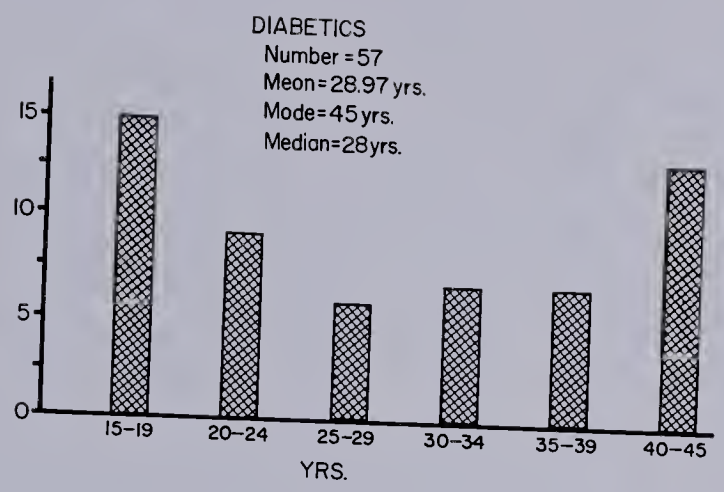
II -Total cross-sectional area of the venules:

Figure XV (page 48) contains the data on mean cross-sectional areas of the non-diabetic conjunctival venules which accompanied the arterioles presented above. Figure XVI (page 49) shows similar data on mean cross-sectional areas of venules of diabetics which also accompanied the arterioles mentioned above. Again, the marked skew to the right is noted and will be corrected later.

III -V/A Ratios in terms of decimal fractions:

The V/A ratios of corresponding venules and arterioles are graphically represented as frequency-distribution curves for non-diabetic (FIG. XVII, page 50) and diabetic samples (FIG. XVIII, page 51). Like the curves for venular and arteriolar cross-sectional area, these two curves are also skewed to the right.

Since a valid statistical comparison of means can not be done unless the distributions are roughly symmetrical, the observations listed in figures XIII - XVIII, (page 46 - 51), were re-plotted after first



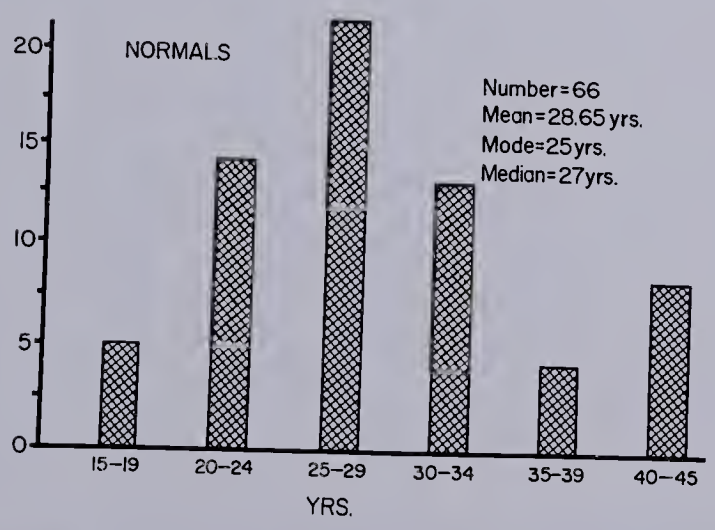


FIGURE XII

HISTOGRAMS SHOWING DATA BY AGE GROUPS FOR THE TWO SAMPLES USED.

N	= 66
Mean	= .50 units^2
S.D.	= .299
Var.	= .089
Range	= .14 - 1.67 units^2
Class width	= .08

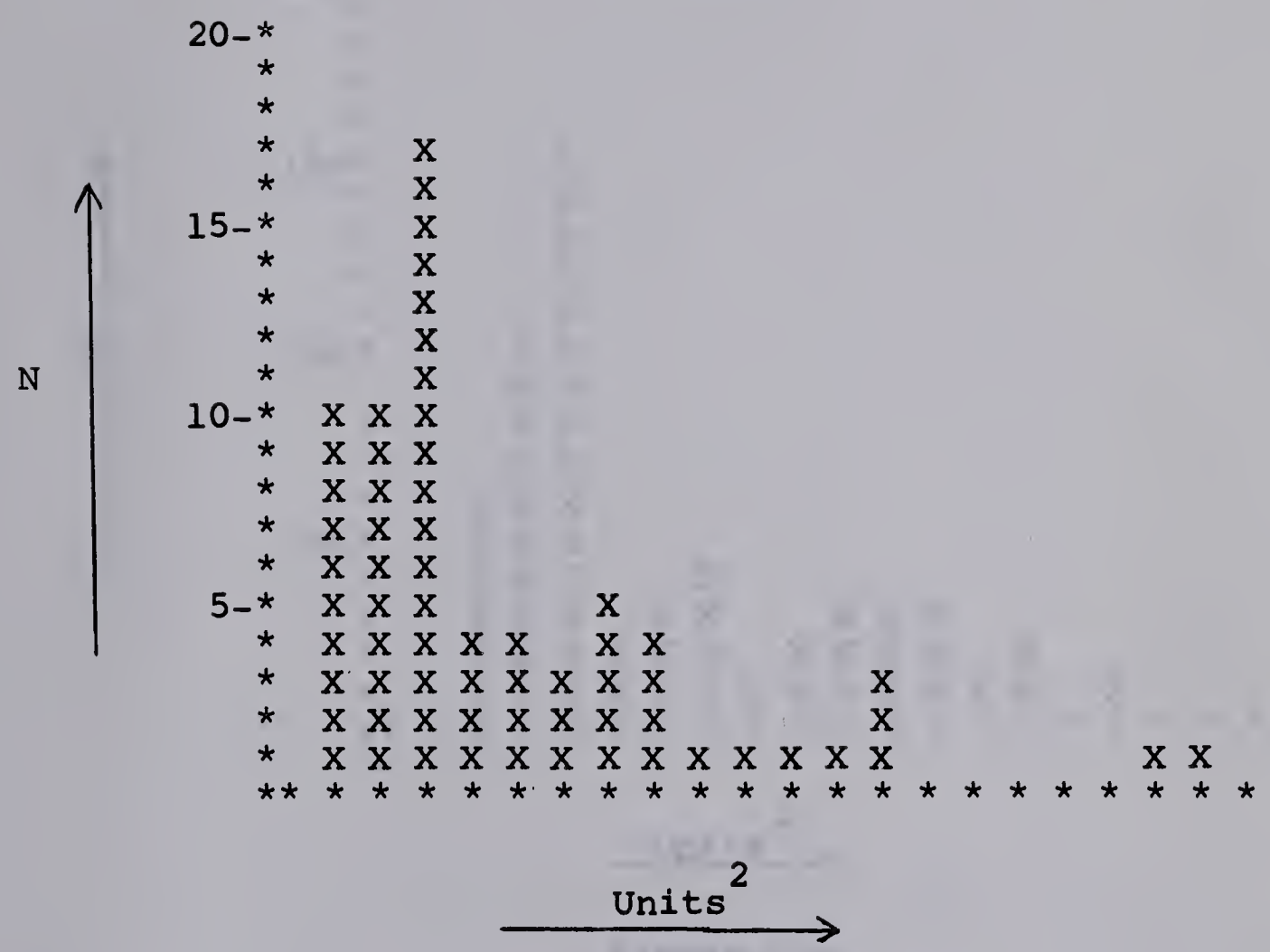
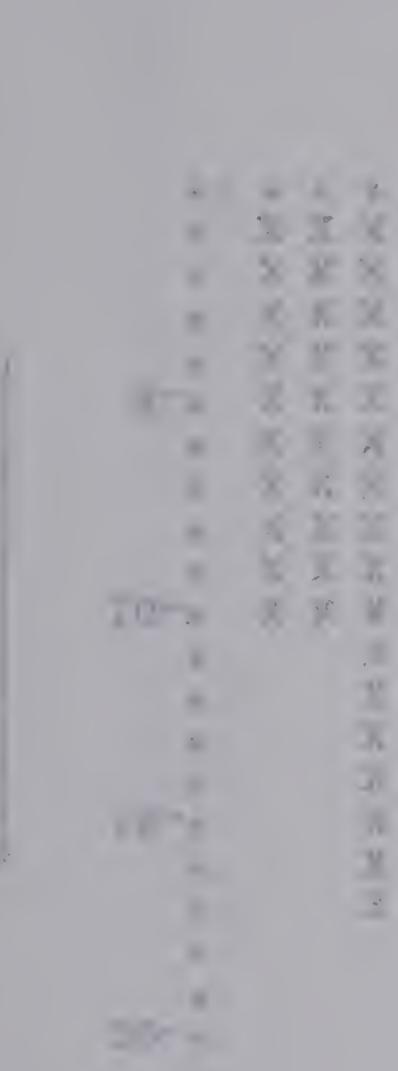


FIGURE XIII

FREQUENCY-DISTRIBUTION OF MEAN CROSS-SECTIONAL AREAS OF ARTERIOLES IN NON-DIABETICS



N =	57
Mean =	.57 units^2
S.D. =	.327
Var. =	.106
Range =	.20 - 1.75 units^2
Class width =	.08

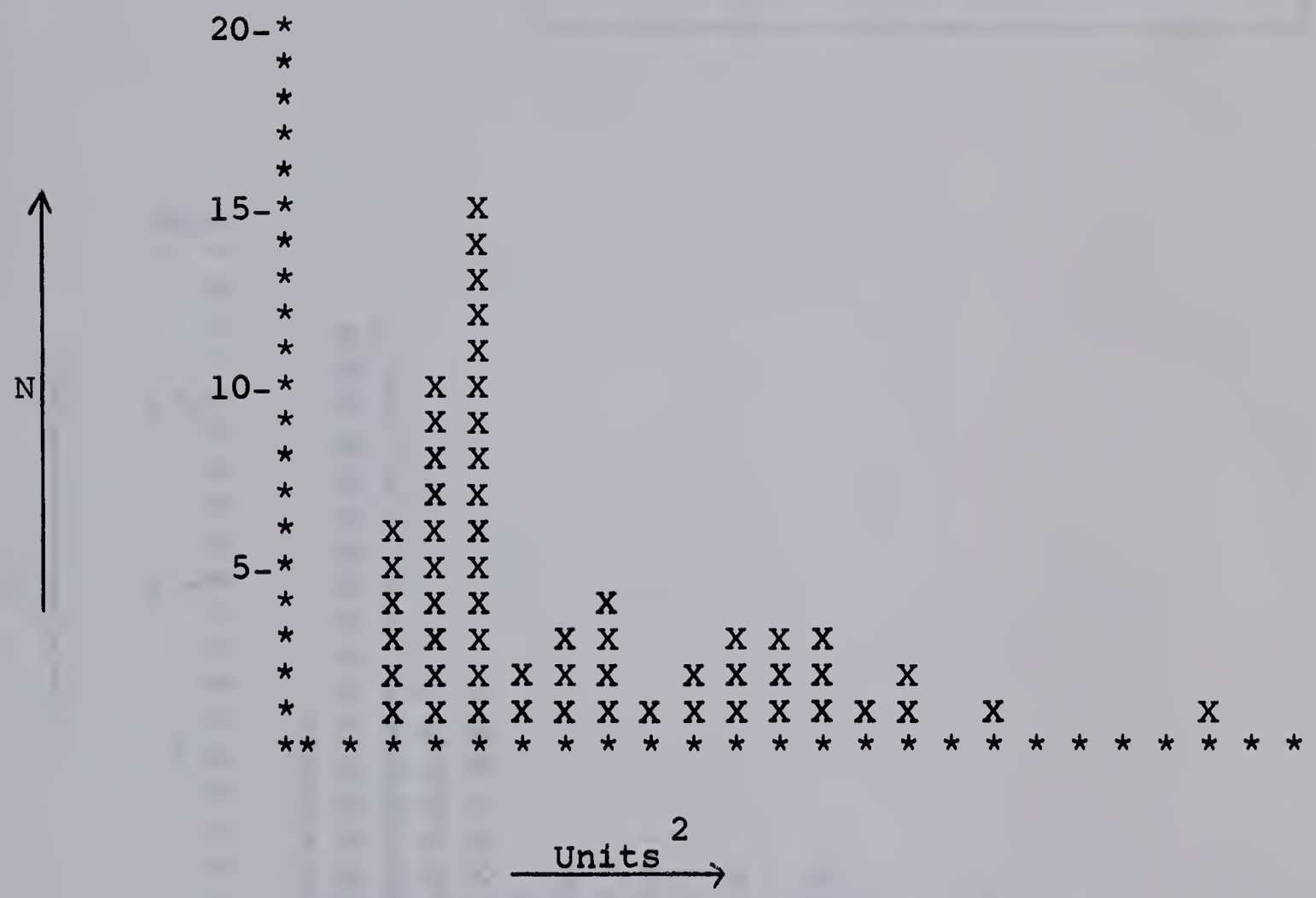
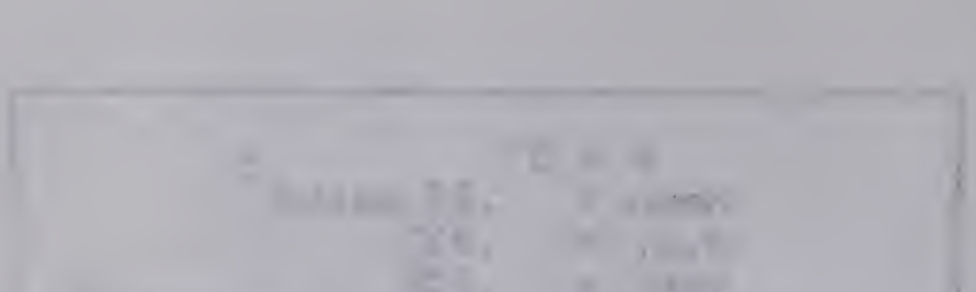


FIGURE XIV

FREQUENCY-DISTRIBUTION OF MEAN CROSS-SECTIONAL AREAS OF ARTERIOLES IN DIABETICS.



N =	66
Mean =	2.30 units^2
S.D. =	1.83
Var. =	3.35
Range =	.46 - 10.19 units^2
Class Width =	.49

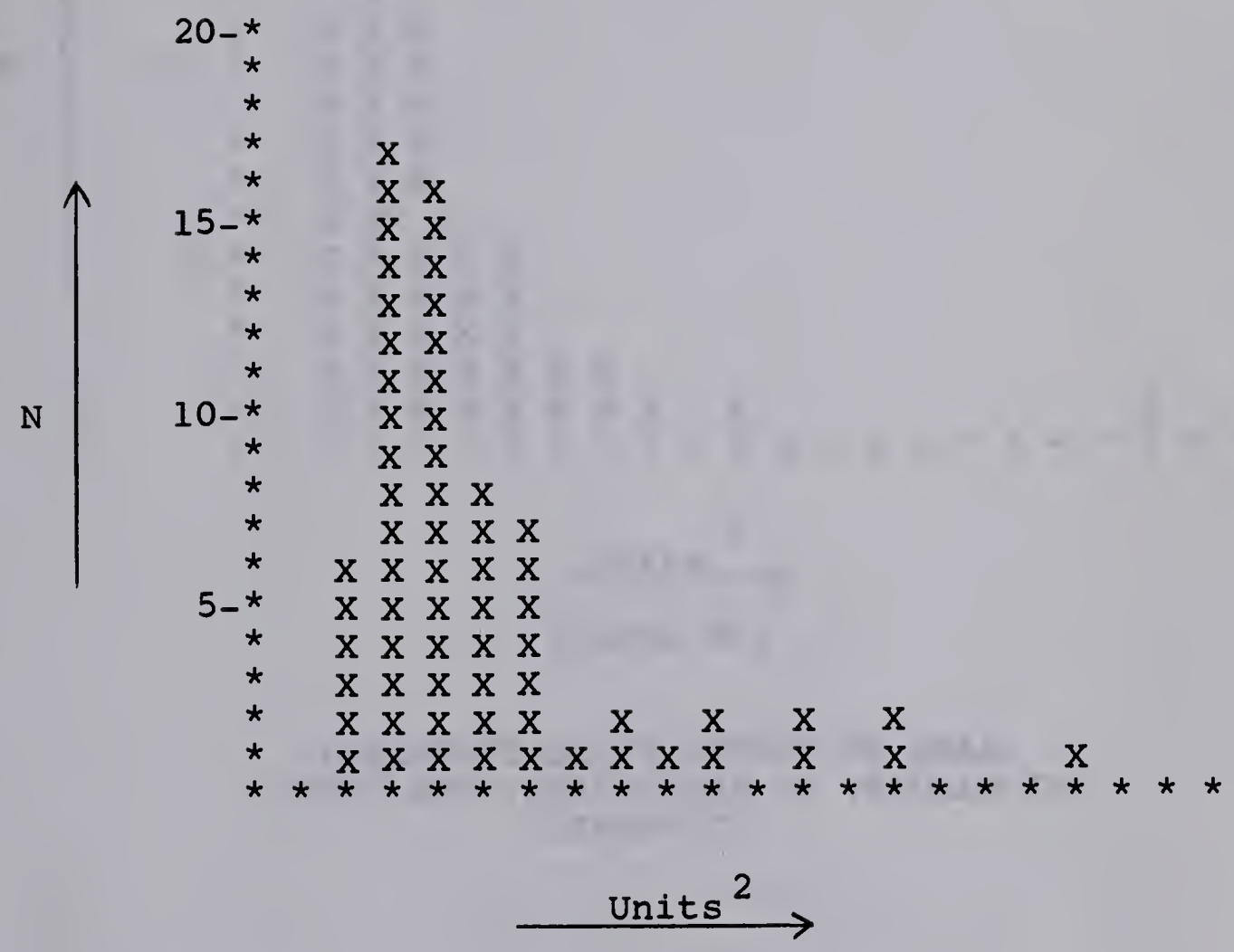
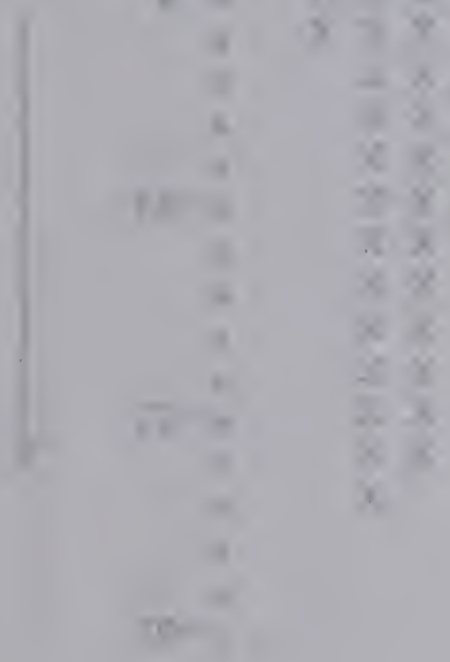


FIGURE XV

FREQUENCY-DISTRIBUTION OF MEAN CROSS-SECTIONAL AREAS OF VENULES IN IN NON-DIABETICS.



N	=	57
Mean	=	2.77 units^2
S.D.	=	2.45
Var.	=	6.01 ^{2}
Range	=	.38 - 16.90 units
Class width	=	.83

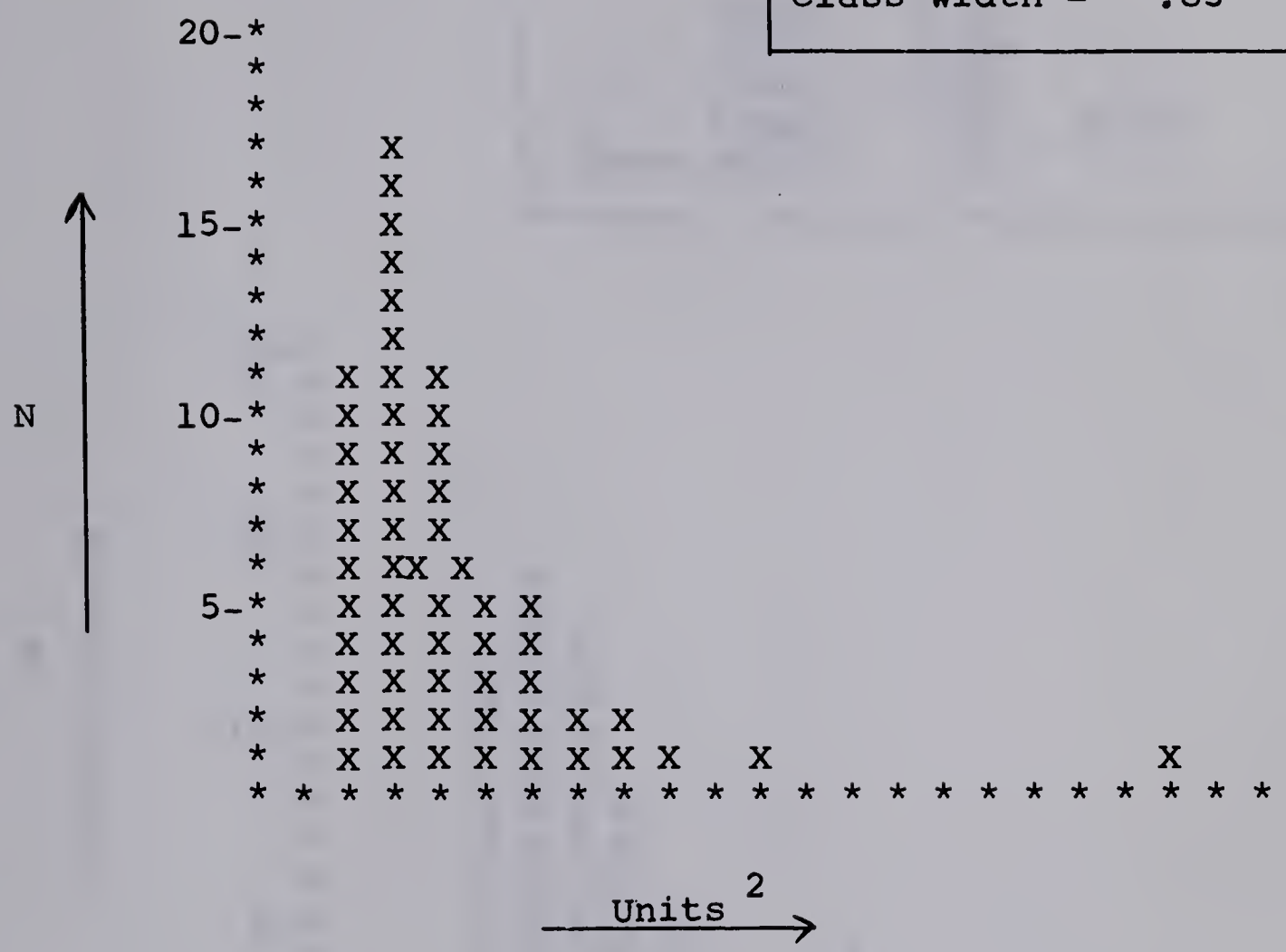


FIGURE XVI

FREQUENCY-DISTRIBUTION OF MEAN CROSS-SECTIONAL AREAS OF VENULES IN DIABETICS.

N	= 66
Mean	= 5.36
S.D.	= 4.19
Var.	= 17.60
Range	= 1.07 - 24.85
Class width	= .12

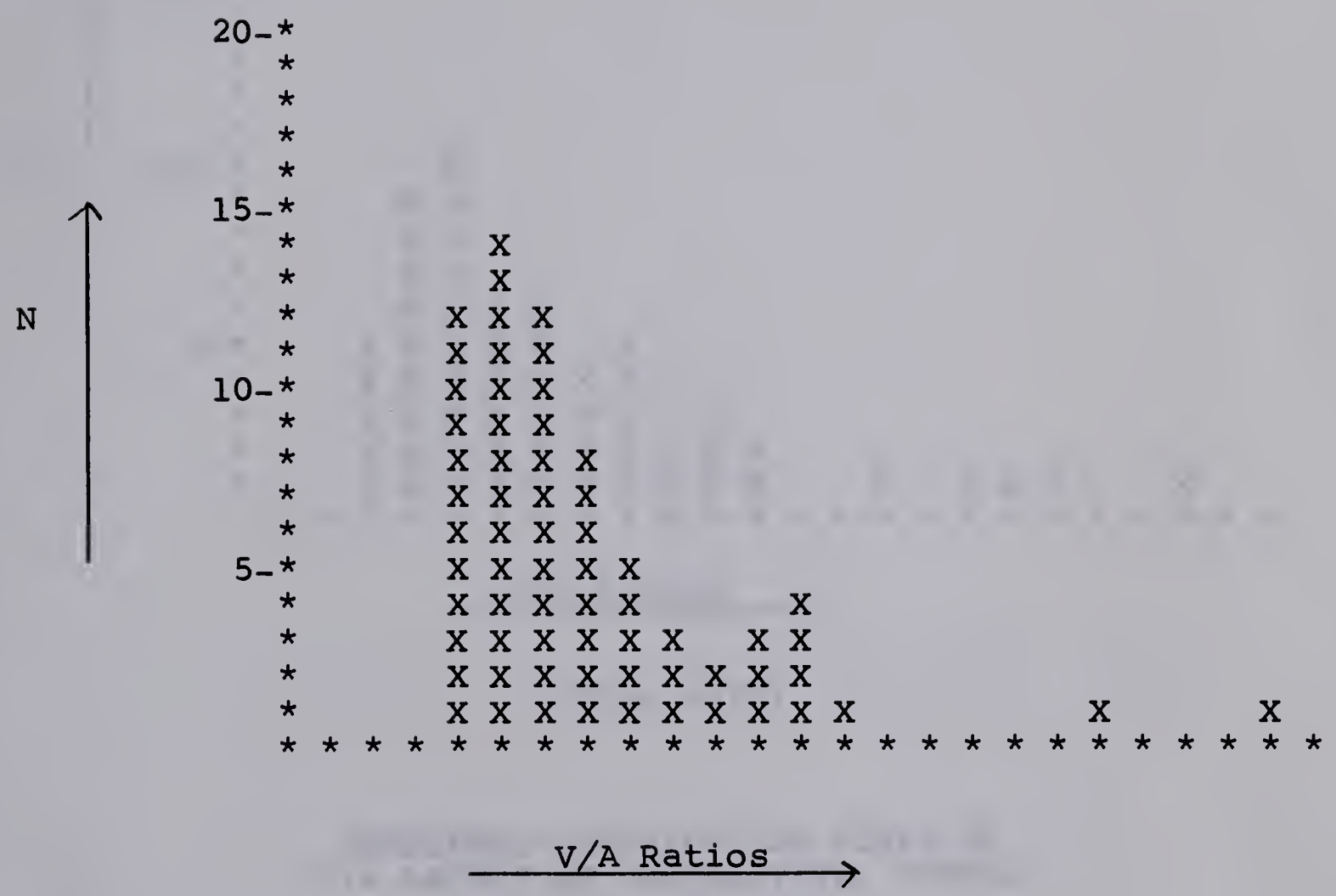


FIGURE XVII

FREQUENCY-DISTRIBUTION CURVE
V/A RATIOS OF CONJUNCTIVAL
VESSEL PAIRS IN NON-DIABETICS.

N	= 57
Mean	= 5.36
S. D.	= 3.67
Var.	= 13.46
Range	= 1.05 - 18.44
Class width	= .87

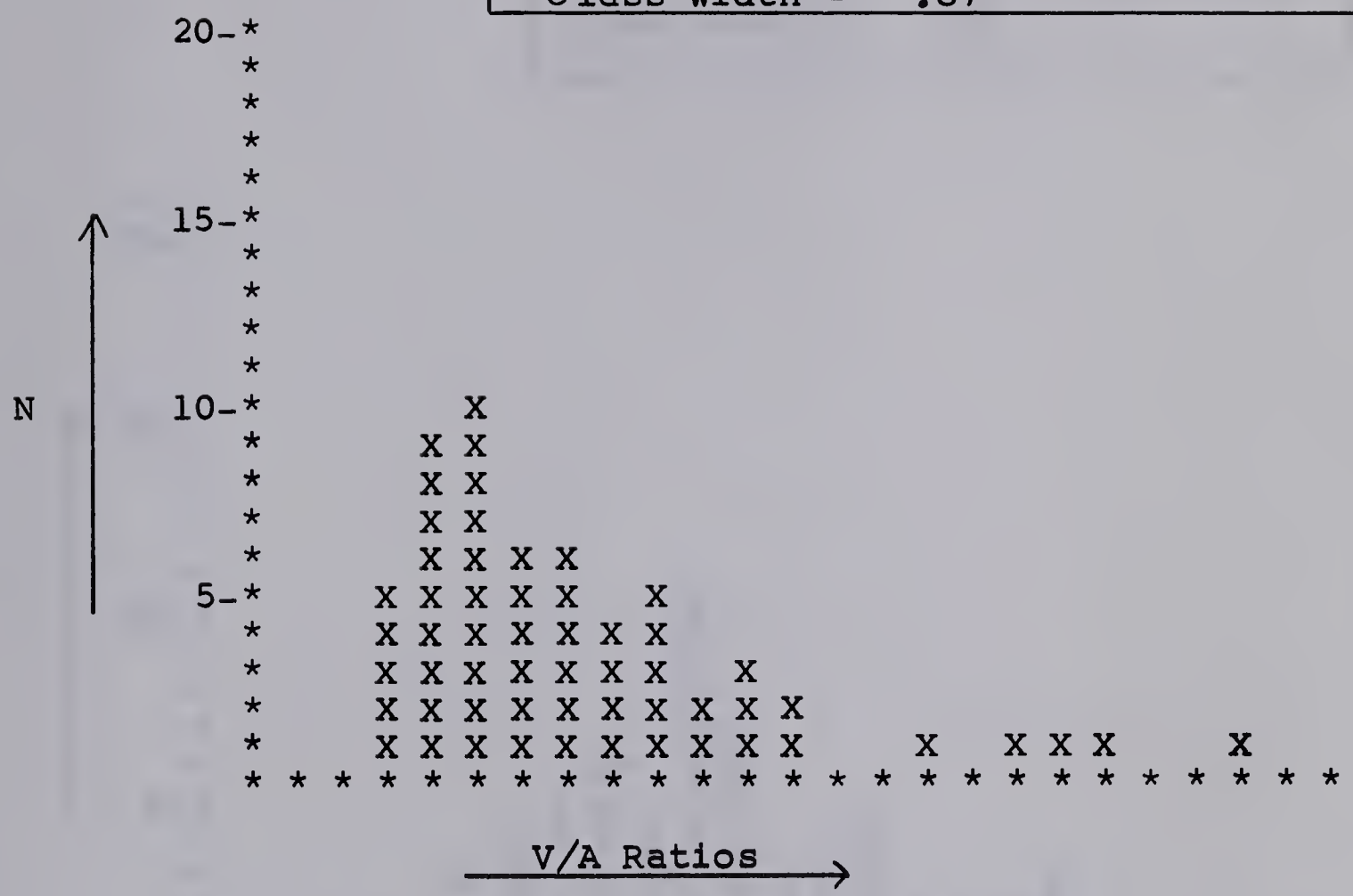


FIGURE XVIII

FREQUENCY-DISTRIBUTION CURVE OF
V/A RATIOS OF CONJUNCTIVAL VESSEL
PAIRS IN DIABETICS

N	= 66
Mean	= .64 units^2
S.D.	= .22
Var.	= .048
Range	= .14 - 1.22 units^2
Class width	= .05

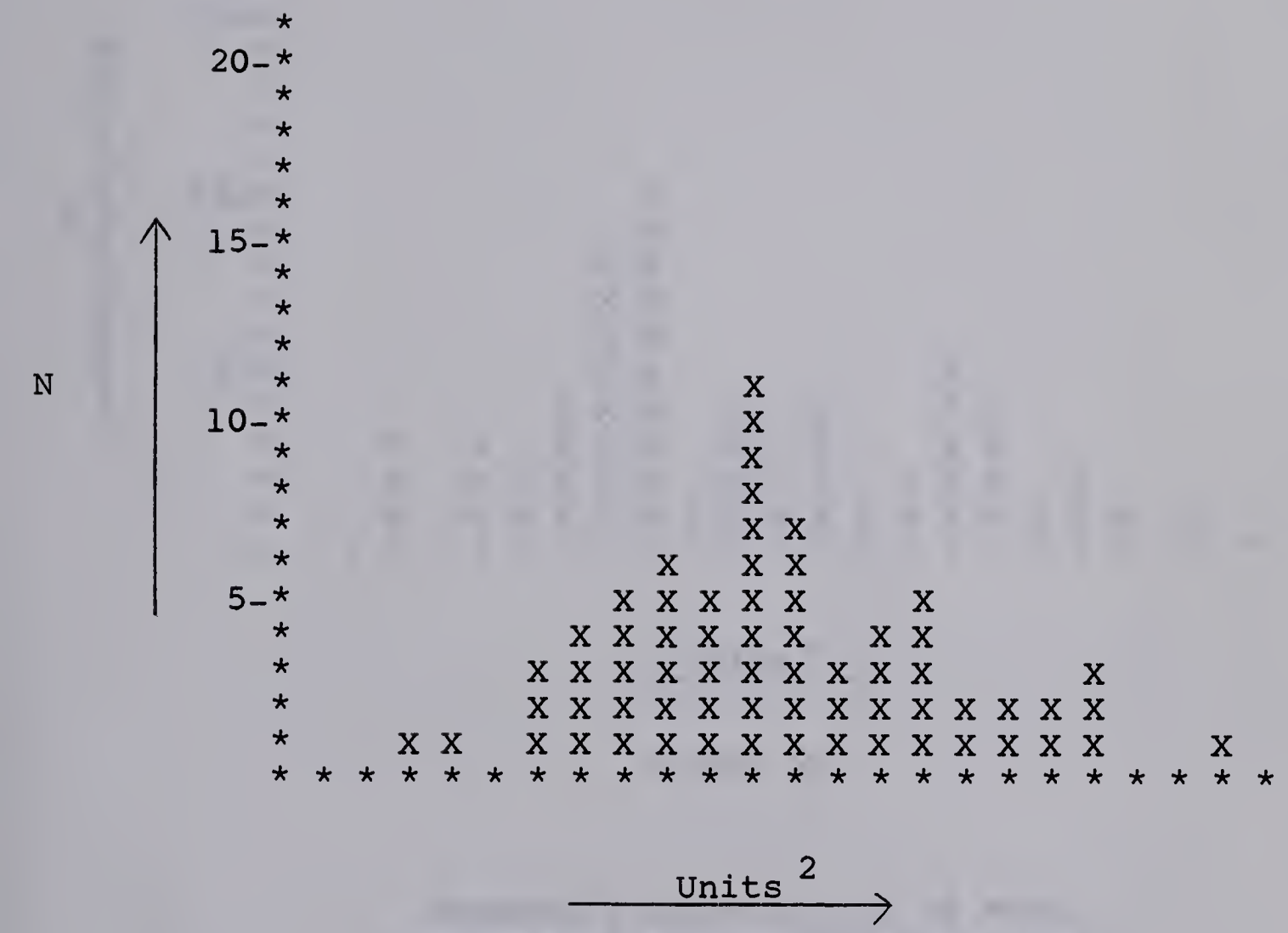
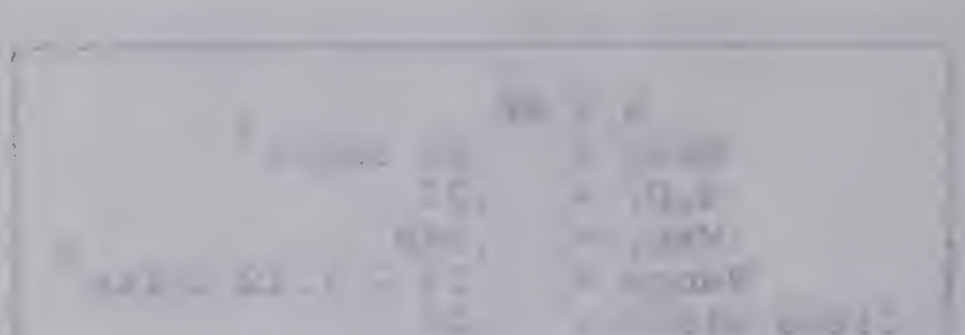
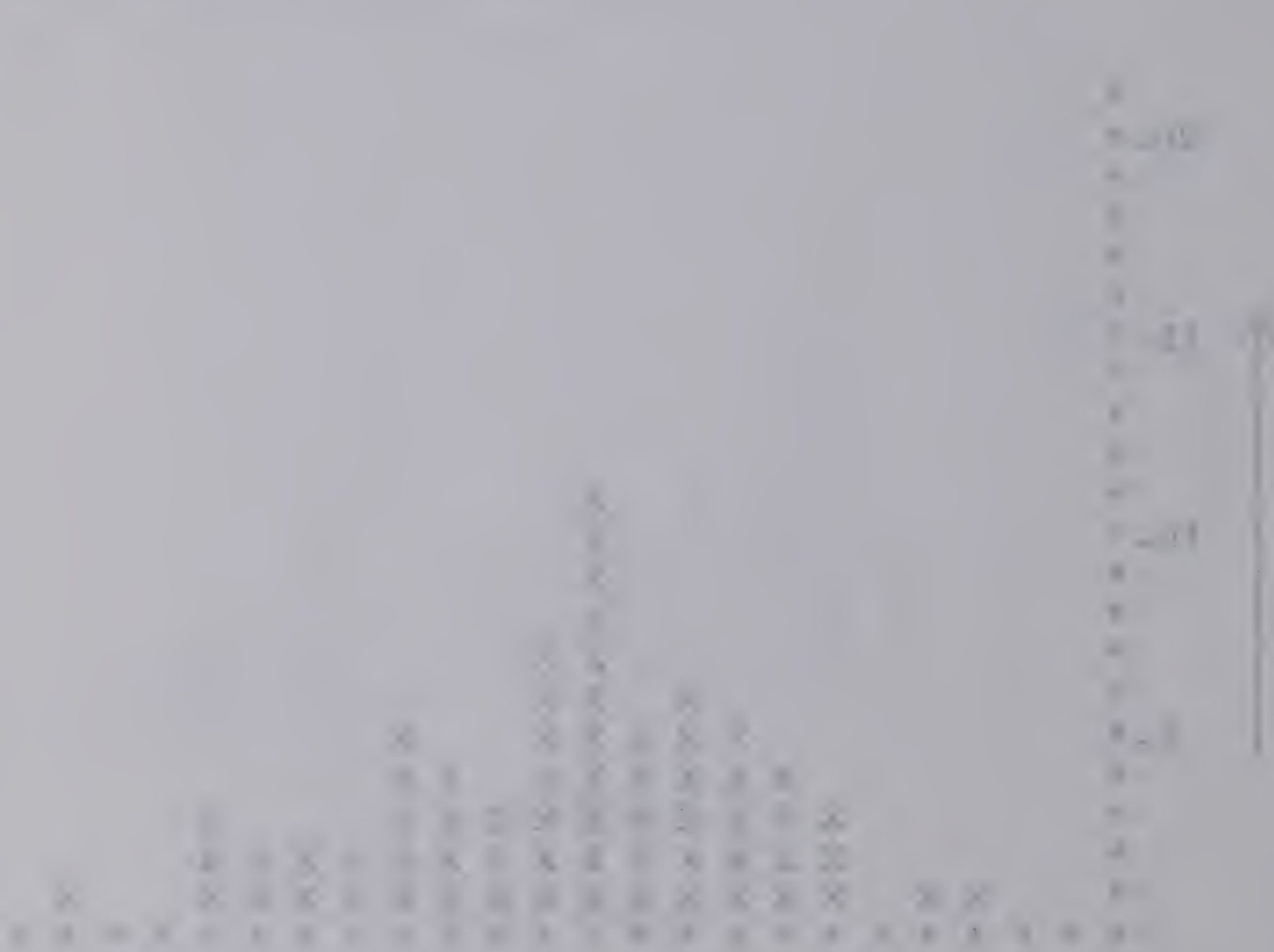


FIGURE XIX

FREQUENCY-DISTRUBUTION OF MEAN ARTERIOLAR CROSS-SECTIONAL AREAS OF ARTERIOLES IN NON-DIABETICS

(Using Log 10 of ten times each Observation.)





N	= 57
Mean	= .70 units^2
S.D.	= .23
Var.	= .050
Range	= .30 - 1.24 units^2
Class width	= .06

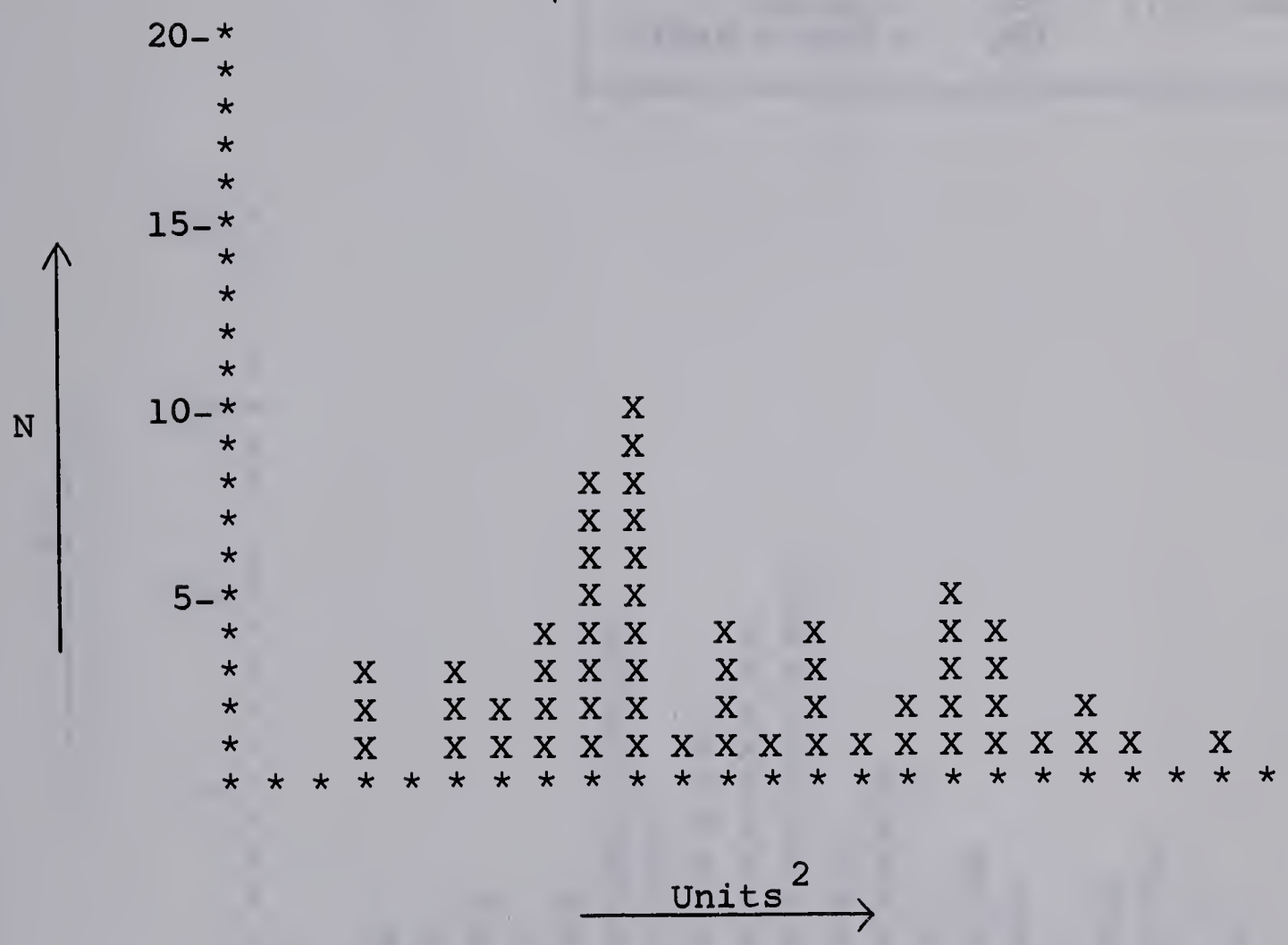
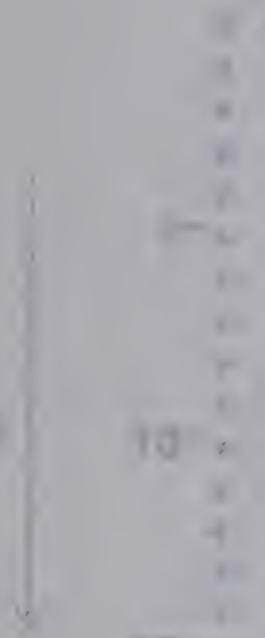


FIGURE XX

FREQUENCY-DISTRIBUTION OF MEAN ARTERIOLAR CROSS-SECTIONAL AREAS OF ARTERIOLES IN DIABETICS

(Using Log_{10} of ten times each Observation)



N	66
Mean	1.27 units^2
S.D.	.27
Var.	.075
Range	.66 - 2.01 units^2
Class width	.07

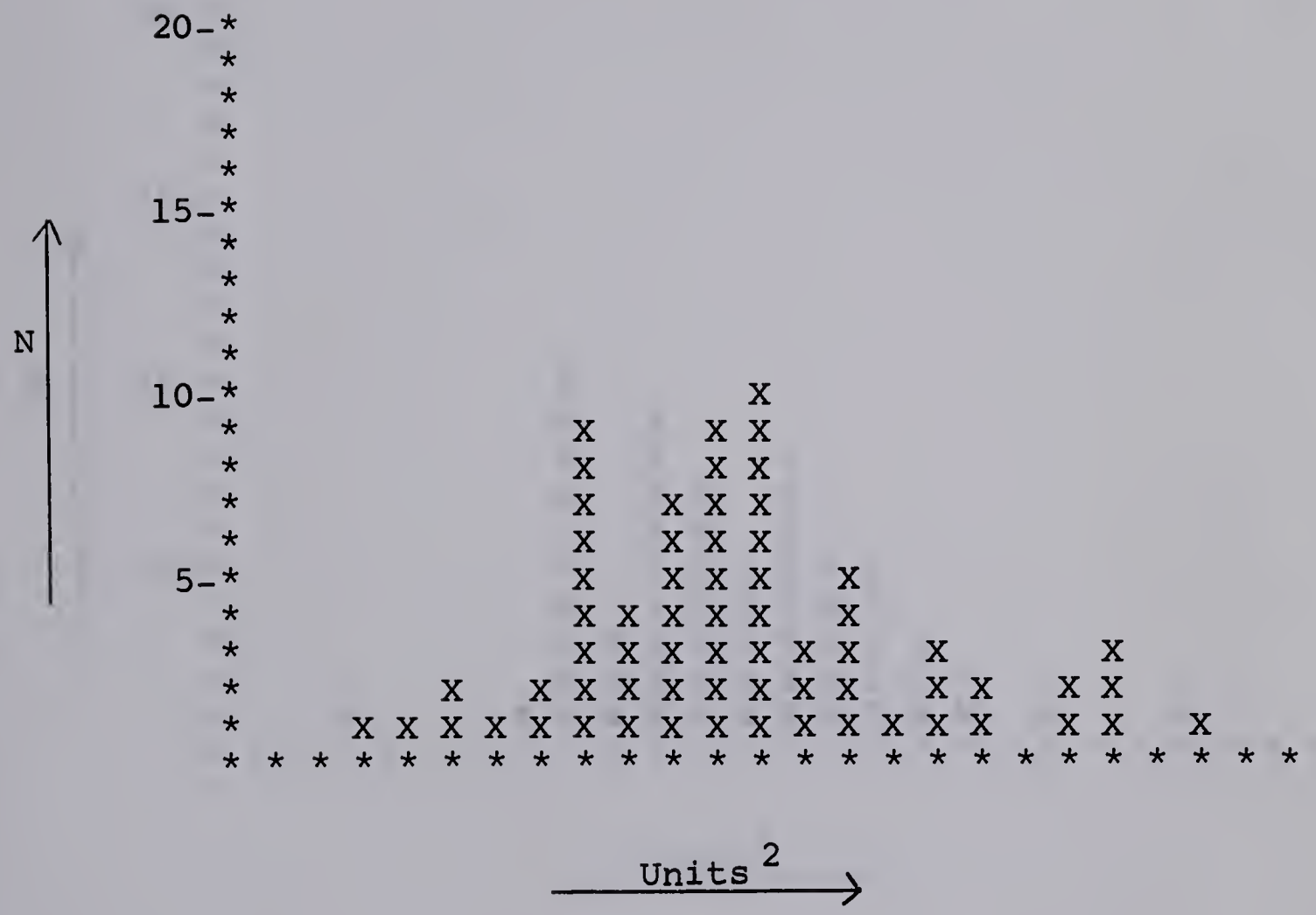
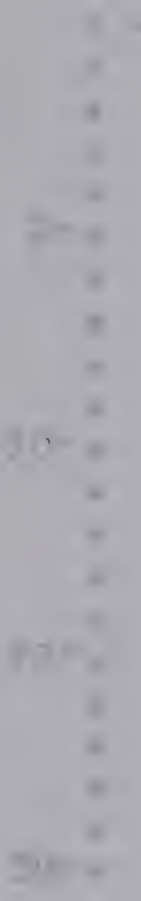


FIGURE XXI

FREQUENCY-DISTRIBUTION OF MEAN VENULAR CROSS-SECTIONAL AREAS OF VENULES IN NON-DIABETICS.

(Using Log_{10} of ten times each observation)



N =	57
Mean =	1.34 units^2
S. D.=	0.28
Var. =	.078
Range =	.58 - 2.23 units^2
Class width =	.08

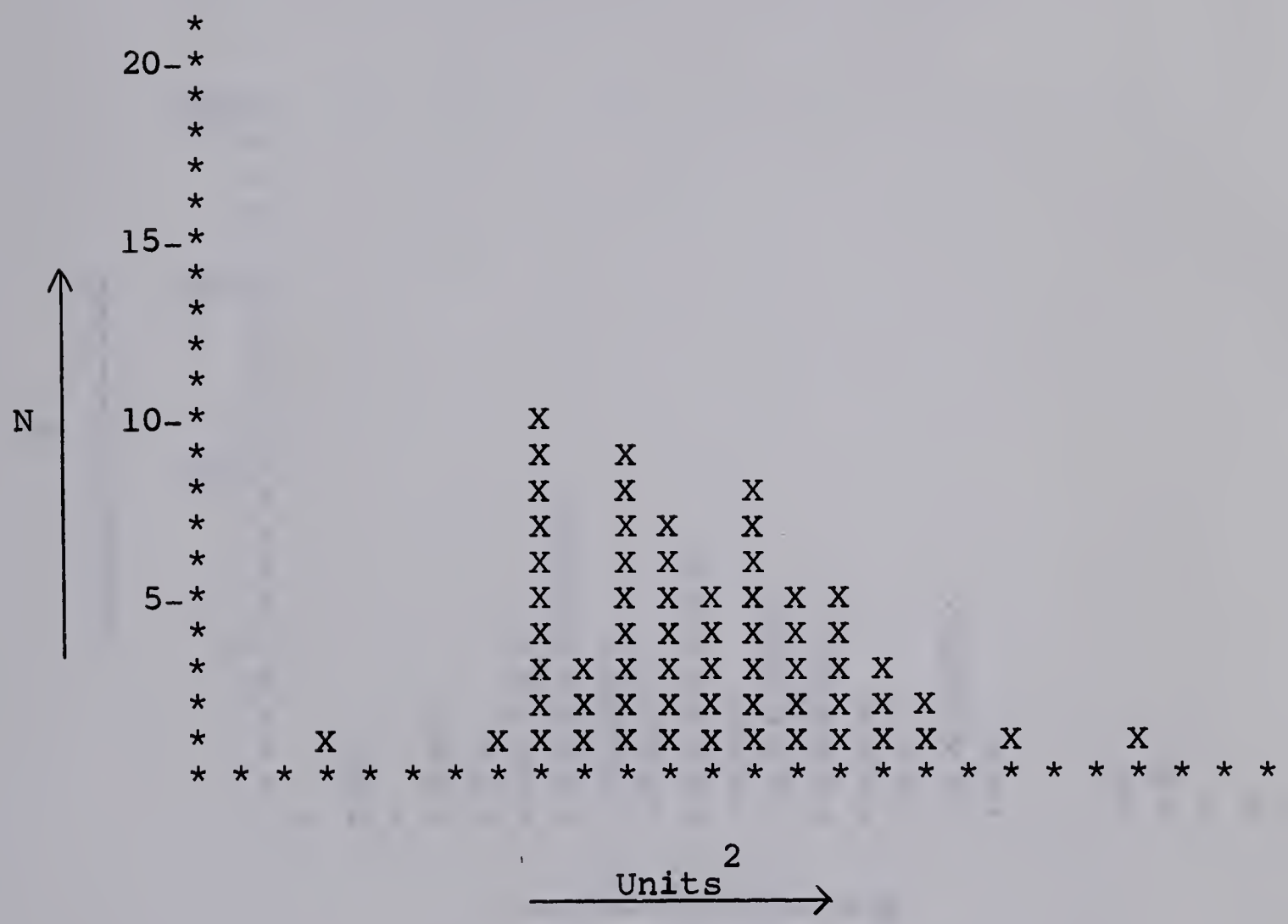
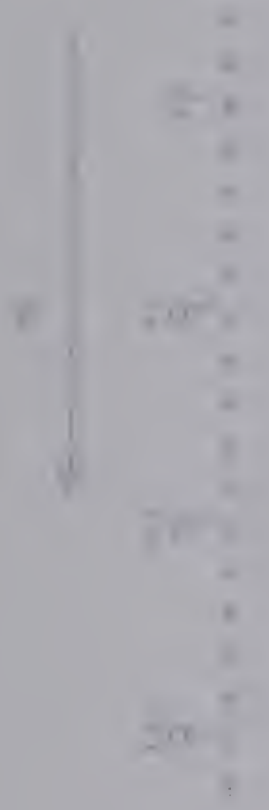


FIGURE XXII

FREQUENCY-DISTRIBUTION OF MEAN VENULAR CROSS-SECTIONAL AREAS OF VENULES IN DIABETICS.

(Using Log_{10} of ten times each observation)



N	66
Mean	1.62
S. D.	0.29
Var.	.065
Range	1.02 - 2.39
Class width	.07

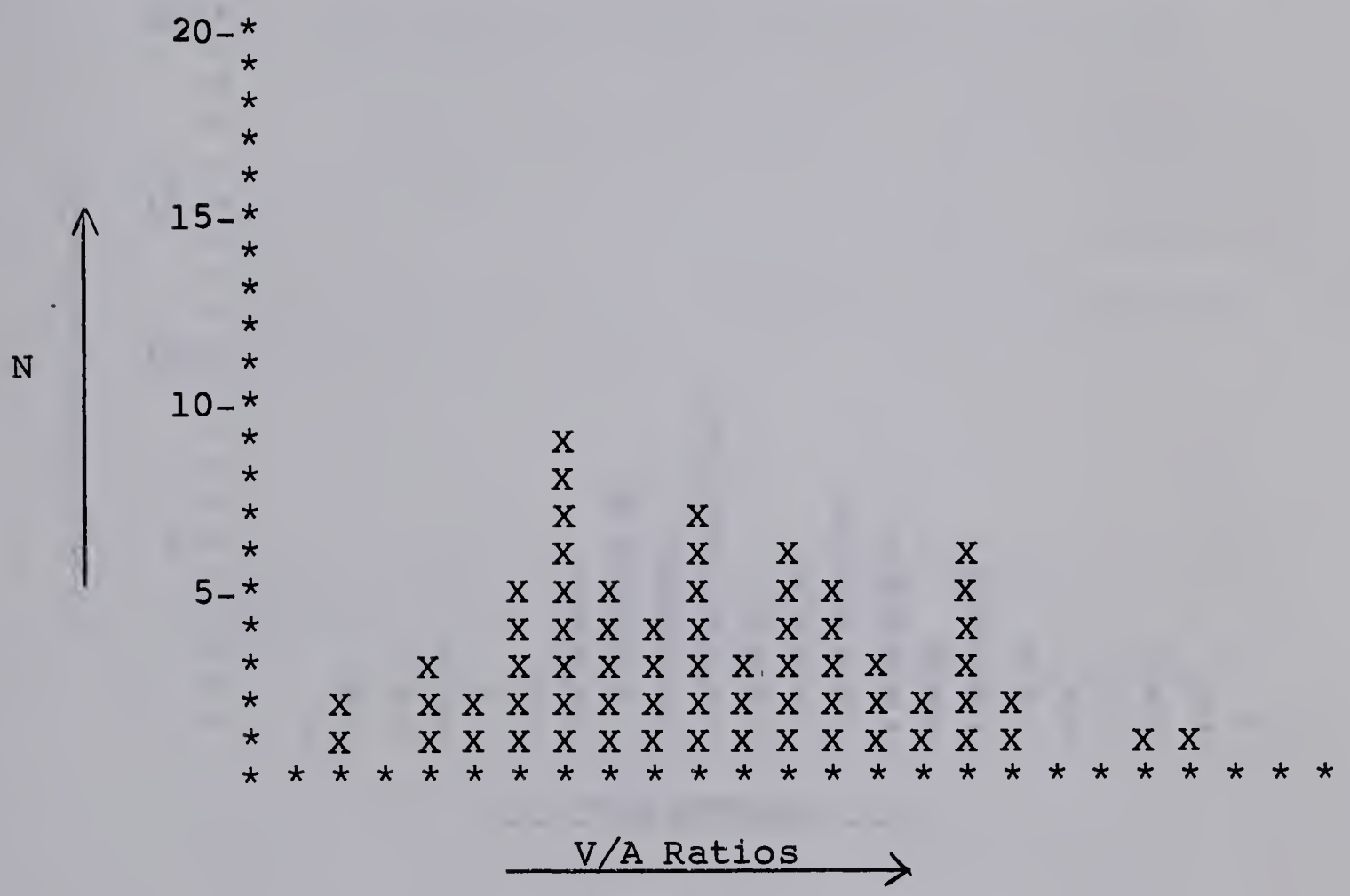


FIGURE XXIII

FREQUENCY-DISTRIBUTION OF V/A RATIOS OF CONJUNCTIVAL VESSEL PAIRS IN NON-DIABETICS.

(Using Log_{10} of ten times each observation)

N	= 57
Mean	= 1.64
S.D.	= 0.27
Var.	= .075
Range	= 1.0 - 2.27
Class width	= .06

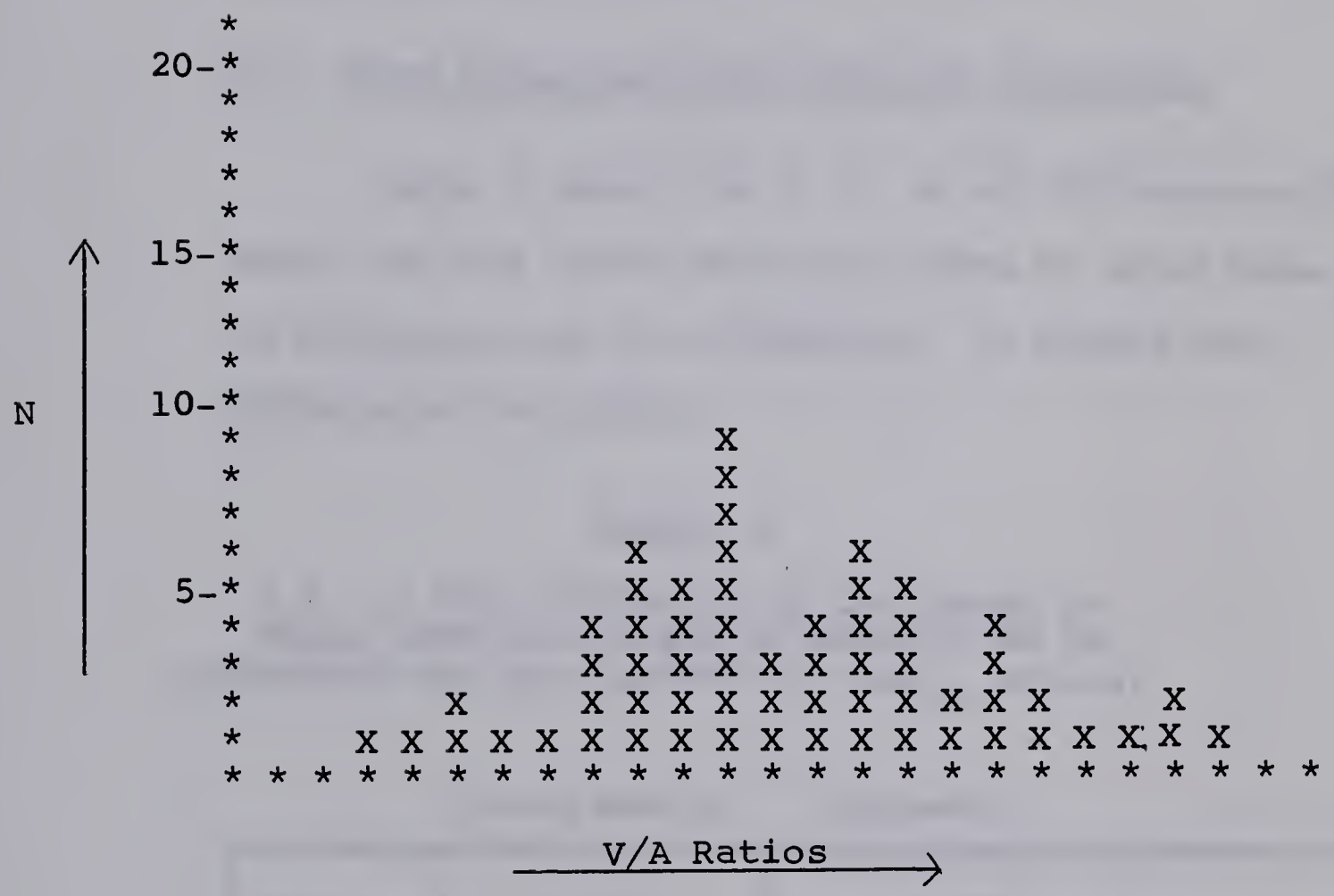


FIGURE XXIV

FREQUENCY-DISTRIBUTION OF V/A RATIOS
OF CONJUNCTIVAL VESSEL PAIRS IN DIABETICS.

(Using Log_{10} of ten times
each observation)

multiplying each variable by ten (to avoid any negative terms) and then taking logarithms to the base 10 for each. This procedure proved to make the distributions roughly symmetrical as can be seen from Figures XIX - XXIV (pages 52 - 57).

STATISTICAL ANALYSIS: (See Appendix A)

(a) Mean Cross-Sectional Area of Arterioles

Table V shows the S. E. of the Difference of Means for the cross-sectional areas of arterioles in diabetics and non-diabetics. No significant difference is evident.

TABLE V

S.E. OF THE DIFFERENCE OF THE MEANS OF CROSS-SECTIONAL AREAS OF ARTERIOLES IN DIABETICS AND NON-DIABETICS (Log_{10} values)

	Non-Diabetic	Diabetic
Mean	0.641	0.699
S.D.	0.221	0.226
Var.	0.048	0.050
S.E. of Difference of Means = .040		
z = 1.45 (A = .4265)		
p = 0.1470		

(b) Mean Cross-Sectional Area of Venules:

Table VI shows the S. E. of the Difference of Means for the cross-sectional areas of venules in diabetics and non-diabetics. No significant difference is evident.

TABLE VI

S.E. OF THE DIFFERENCE OF THE MEANS OF CROSS-SECTIONAL AREAS OF VENULES IN DIABETICS AND NON-DIABETICS (Log_{10} values)

	Non-diabetic	Diabetic
Mean	1.269	1.345
S. D.	0.274	0.280
Var.	0.075	0.078
S. E. of Difference of Means = .050		
		z = 1.52 (A = .4357)
		p = 0.1286

(c) Mean V/A Ratios of Conjunctival Vessel Pairs:

Table VII shows the S. E. of the Difference of Means for the V/A ratios of conjunctival vessel pairs in diabetics and non-diabetics. The results indicate that no significant difference is present.

TABLE VII

S.E. OF THE DIFFERENCE OF THE MEANS OF V/A RATIOS OF CONJUNCTIVAL VESSEL PAIRS IN DIABETICS AND NON-DIABETICS. (Log_{10} Values)

	Non-Diabetics	Diabetics
Mean	1.628	1.643
S. D.	0.292	0.274
Var.	0.075	0.078
S.E. of Difference of Means = 0.051		
	z = 0.29 (A = 0.1141)	
	p = 0.7718	

IV - Intra-Individual Variations of V/A Ratio:

Twenty-two photographs from the Non-Diabetic sample and twenty photographs from the Diabetic sample contained two pairs of vessels which were measurable. In order to see whether any significant difference existed between two V/A ratios in the same person the Wilcoxon Test for the Paired Case was utilized. (See Appendix A, pages 83 - 90)

TABLE VIII

TABLE SHOWING THE PROBABILITY OF DIFFERENCES IN V/A RATIOS OF VESSEL PAIRS IN THE SAME INDIVIDUAL OCCURRING BY CHANCE, IN DIABETICS AND NON-DIABETICS.

	Non-diabetics	Diabetics
No. subjects with two pairs vessels	22	20
Positive ranks	171	47
Negative ranks	82	163
Probability that difference might have occurred by chance alone	p = 0.147	p = 0.030

Table VIII indicates that the difference between two V/A ratios in the same person amongst Non-Diabetics is not statistically significant and the difference between two V/A ratios in the Diabetic sample is not significant at a level of significance of P = .01.

DISCUSSION:

The bulbar conjunctiva was chosen for study because it is easily accessible, appears to be embryologically and anatomically related to the smaller blood vessels of the subcutaneous tissues and can be considered representative of this part of the vascular bed and it permits inspection of a statistically valid sample of circulating blood (15,27).

Photographic Difficulties:

In attempting to photograph a convex mucous membrane which is constantly moving, many difficulties have to be overcome. The prisms of the microscope absorb a great deal of light before it reaches the film and a flash of sufficient intensity, but still innocuous to the eye, must be used. We found a 3½" close-up ring strobe with a light-output of 30 watt-seconds, placed 6 - 8" from the eye provided adequate illumination for exposing a fast film. In attempting to render the panchromatic film we used, relatively orthochromatic, a compromise had to be reached between the amount of red light and the total amount of light filtered out. The palest blue filter was used (Kodak 82A) which filtered out about 50 percent of the red light while still allowing enough light of other wave

lengths to be transmitted, thus allowing adequate exposure of the film.

The electronic flash was of 1/1000 sec. duration and was synchronized with the reflex shutter. At this speed, the physiological end-point nystagmus which is present when the eyes look far off in one direction is of no consequence.

Our greatest problem with the photography was avoiding glare from the moist conjunctiva. Since we were photographing a moist, convex surface, some glare was inevitable. It could be minimized, however, by having the flash at right angles to an imaginary tangent to the bulbar conjunctiva, in the region where the conjunctiva was in focus.

The focussing light of the unit emitted a beam of light which was perfectly cold so that no heat-induced changes were possible in the conjunctival vasculature.

By the nature of the system used, no diaphragming was possible. Since the surface photographed was convex, only the middle portion of the film was in perfect focus.

A relatively fast film was used and for this reason the grain of the picture is somewhat coarse. Maximum contrast was obtained by over-developing the film.

Discussion of Results:

No significant difference was found between the mean arteriolar cross-sectional area, mean venular cross-sectional area or V/A ratios between diabetic and non-diabetic sample groups. This is clearly shown in tables V to VII (pages 58 - 60).

These results differ from some others reported previously in the literature for the age group concerned. Ditzel (23, 24) found an altered V/A ratio in 48% of diabetic subjects between the ages of 35 and 55. Bech (12), studying 68 diabetics and 24 controls under fifty years of age found that the difference of mean V/A ratios between the diabetic and normal samples was significant. (The value of "t" lying between the 95 - 97.5 percentiles).

This study showed no significant variation between two V/A ratios of different vessel pairs in the same person whether he was diabetic or non-diabetic (Table VIII, page 61). Bech found a significant variation between two V/A ratios in the same person in his non-diabetic sample (12).

Analysis of Methodology:

Since the results in this work differ from those

of Ditzel and Bech, it is necessary to attempt to find some reason for these experimental differences. Certain differences in methodology will subsequently be discussed:

(a) - Position of Subject:

In this study, the patient was seated erect at a slit-lamp table as were the patients of Bech. Ditzel did his photography with the patient in the supine position (23, 24), although in one publication he mentions doing some observations with the patient erect (27). If a vessel were to have some intrinsic defect in the wall with loss of elasticity, the conjunctival congestion which would result with the patient in the supine position might be exaggerated. This defect in vessel wall elasticity would not be noticed in the erect position.

(b) - Measurement of the Vessels:

The measurement of vessels is a procedure wrought with many error factors. Both Ditzel (23) and Bech (12) used ocular micrometers for measuring the diameters of vessels. This involves viewing a vessel, either *in vivo* or recorded on a photograph, with the aid of a measuring microscope and attempting to superimpose the image of an ocular graticle on top of the vessel, in order that some measurement of vessel diameter might be obtained.

In this study, photographs of constant magnification were projected onto a matte white screen and measurements were than taken directly using calipers which were then placed on to a fine scale which was subsequently read with the aid of a viewing glass. (FIG. X, page 39). We felt that this was a more accurate method of measuring vessels. In order to minimize the human error factor in taking the actual measurements, TEN measurements of each vessel were taken and the mean of these was then taken as the measurement for that particular vessel. Bech took five measurements of each vessel while Ditzel took only three per vessel.

(c) Mean Cross-Sectional Areas:

As was mentioned previously (page 40), the cross-sectional area is a better measure of vessel size than is diameter. Figure XI (page 41) shows the fallacy of adding vessel diameters rather than cross-sectional areas. Ditzel and Bech both used straight vessel diameters in their calculations.

(d) Subjective Observations:

No attempt was made to evaluate microvascular changes which could not be measured mechanically. It is the opinion of the author that subjective impressions

are of questionable value when trying to make scientific comparisons. Ditzel (23) mentioned conjunctival edema and capillary elongation as two subjective changes which were more common in diabetics than normals. The value and validity of observations of this nature are questionable.

(e) General Errors in Analysis:

The bulbar conjunctiva is constantly being subjected to the trauma of the sun and wind, local trauma due to rubbing and foreign substances (particulate matter like dust) and ocular infections (15, 20). For this reason any changes found in the conjunctiva should be interpreted with some caution. Any reports of conjunctival microaneurysms in diabetics have all agreed that they are found only in that part of the bulbar conjunctiva which is exposed to the elements.

Ditzel (23) was convinced that there were changes in the conjunctiva characteristic of diabetes. Bech (12), to a limited degree, substantiated these findings. Other workers have not been impressed by any changes in the conjunctival vessels in diabetes. C. A. G. Cook (7) in a preliminary study, failed to confirm the conclusions of Ditzel and his co-workers. Hintz (39), in a recent publication from Poland, also was unable to find any specific changes in

diabetic conjunctivae. Ashton (8) is also of the opinion that changes in the conjunctiva are non-specific.

Microaneurysms which have been reported in the conjunctiva in diabetes (34, 48, 58) and a variety of disease differ from the typical diabetic microaneurysms of the retina both in size, shape and number.

The incidence of microaneurysms found by these same authors in the conjunctivae of diabetics, bear no relationship to the duration of the disease as do retinal microaneurysms.

This author was unable to find microaneurysms in a significant number of diabetics which is in keeping with the findings of Ashton (6), Ditzel (23) and Cook (20).

On the whole it is probably the case that no gross changes specific to diabetes occur in the conjunctival vessels although there is microscopic evidence, in the form of thickened basement membranes of small vessels throughout the body, for generalized microvascular disease.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Summary:

The purpose of this study was to investigate the conjunctival microvasculature in diabetic and non-diabetic populations in an attempt to corroborate the previous claims of Ditzel.

Fifty-seven diabetics and sixty-six non-diabetic patients were used in this study. The diabetics fulfilled a number of given criteria (Table II, page 22) as did the controls (Table III, page 23). Diabetes was ruled out in the control population using a two-hour blood glucose determination after a standard meal containing 200 gm. Carbohydrate (Table IV, page 24).

Photography was carried out only between the hours of ten and twelve in the morning since this was the supposed period of maximal venular dilatation reported by others (35). The pictures were taken of the temporal aspect of the bulbar conjunctiva of the right eye in each subject. Three photographs, containing at least one pair of vessels were taken at random in the region midway between limbus and outer canthus.

The resulting negatives were projected on to a matte white screen situated five feet distant from

the slide. Ten measurements were then taken of each venule and arteriole which comprised a vessel pair. The diameter measurements were then converted to total cross-sectional area, a more accurate measurement of vessel size.

Statistical analysis was performed using the Standard Error of the Difference between Means to compare: mean venular cross-sectional area, mean arteriolar cross-sectional area and mean V/A ratios between the Diabetic and Non-Diabetic samples.

The Wilcoxon Test for the Paired Case was used to evaluate any difference between two V/A ratios in the same individual, either diabetic or non-diabetic.

Conclusions:

On the basis of the analysis used and within the limitations of this study the following conclusions appear to be justified:

i. No difference of any significance, was found between mean cross-sectional areas of arterioles in diabetic and non-diabetic samples.

ii. No significant difference was found between mean cross-sectional areas of venules in diabetic and non-diabetic samples.

iii. No significant difference was found between mean V/A ratios for vessel pairs in diabetic and non-diabetic samples.

iv. No significant difference was found between mean V/A ratios of two vessel pairs in the same person in the diabetic sample.

v. No significant difference was found between mean V/A ratios of two vessel pairs in the same person in the non-diabetic sample.

No significant difference was found between any of the measurable parameters of the conjunctival microcirculation in representative samples of Diabetics and Non-Diabetics.

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APPENDIX A

STATISTICAL TREATMENT

Distribution of the Difference between two Sample Means: (3,40)

Consider two populations X and Y. If all possible samples of n variates were drawn from each population, we would get a group of sample means for each population which might be represented $\bar{X}_1$, $\bar{X}_2$, $\bar{X}_3$, $\bar{X}_4$, ...$\bar{X}_n$ for the first population and $\bar{Y}_1$, $\bar{Y}_2$, $\bar{Y}_3$, $\bar{Y}_4$, ...$\bar{Y}_n$ for the second population.

If n exceeds 30, the means of all the samples from each population will have normal frequency-distribution curves with standard deviations. The difference between sample means may be measured using an expression called:
The Standard Error of the Difference Between Means
and is given by the formula:

$$\sigma_{\bar{x} - \bar{y}} = \sqrt{\frac{\sigma_x^2}{n_x} + \frac{\sigma_y^2}{n_y}}$$

If the difference between two sample means is greater than two times the standard error derived from the above formula, then the differences are significant, probably not arising by chance.

Since the sample number of the Diabetic population was 57 and that of the Non-Diabetic population was 66 (both numbers well over 30), it was decided that the Standard Error of the Differences of the Means be employed to compare differences of sample means:

(1) Between mean arteriolar cross-sectional areas of conjunctival arterioles sampled in Diabetic and Non-Diabetic populations.

(2) Between mean venular cross-sectional areas of conjunctival venules sampled in Diabetic and Non-Diabetic populations.

(3) Between V/A ratios of conjunctival vessel pairs in Diabetic and Non-Diabetic populations.

The calculations on the following three pages illustrate the three comparisons which were performed as outlined above.

CROSS-SECTIONAL AREA OF ARTERIOLES:

Non-Diabetics (Table XVIII) Diabetics (Table XIX)

$\bar{x} = 0.641$ $\bar{y} = 0.699$

$\sigma x = 0.2210$ $\sigma y = 0.2255$

$n_x - 66$ $n_y = 57$

Difference of the means = 0.058

$$\sigma_{\bar{x} - \bar{y}} = \sqrt{\frac{x^2}{n_x} + \frac{y^2}{n_y}}$$

$$= \sqrt{\frac{(0.221)^2}{66} + \frac{(0.226)^2}{57}}$$

$$= \sqrt{.0016}$$

$$= .040$$

$$\text{Now } z = \frac{\bar{x} - \bar{y}}{\sigma}$$

$$= \frac{.058}{.040}$$

$$= 1.45 \quad (A = .4265)$$

$$P = .1470$$

This figure is not significant when P = .05 is taken as the level of significance.

$$= \frac{0.040}{0.016}$$

$$\text{MOE } E = \frac{Z}{\bar{x} - \bar{y}}$$

$$= 0.040$$

$$= \sqrt{\quad}$$

$$= \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

$$\text{C.I.} = \bar{x} - \bar{y} = \sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$$

CROSS-SECTIONAL AREA OF VENULES

Non-Diabetics (Table XX)	Diabetics (Table XXI)
$\bar{x} = 1.269$	$\bar{y} = 1.345$
$\sigma x = 0.2736$	$\sigma y = 0.2803$
$n_x = 66$	$n_y = 57$

Difference of means = 0.076

$$\sigma_{\bar{x} - \bar{y}} = \sqrt{\frac{\sigma_x^2}{n_x} + \frac{\sigma_y^2}{n_y}}$$

$$= \sqrt{\frac{(0.274)^2}{66} + \frac{(0.280)^2}{57}}$$

$$= \sqrt{.0025}$$

$$= 0.050$$

$$\text{Now } z = \frac{\bar{x} - \bar{y}}{\sigma}$$

$$= \frac{0.076}{0.050}$$

$$= 1.52 \qquad (A = .4357$$

$$P = .1286$$

This figure is not significant when P = .05 is taken as the level of significance.

V/A RATIOS OF CONJUNCTIVAL VESSEL PAIRS:

Non-Diabetics (Table XXII)	Diabetics (Table XXIII)
$\bar{x} = 1.628$	$\bar{y} = 1.643$
$\sigma x = 0.292$	$\sigma y = 0.274$
$n_x = 66$	$n_y = 57$

Difference of means = 0.015

$$\sigma_{\bar{x} - \bar{y}} = \sqrt{\frac{\sigma_x^2}{n_x} + \frac{\sigma_y^2}{n_y}}$$

$$= \sqrt{\frac{(0.292)^2}{66} + \frac{(0.274)^2}{57}}$$

$$= \sqrt{.0026}$$

$$= .051$$

$$\text{Now } z = \frac{\bar{x} - \bar{y}}{\sigma}$$

$$= \frac{0.015}{0.051}$$

$$= 0.29 \qquad (A = .1141)$$

$$P = .7718$$

This figure is not significant when P = .05 is taken as the level of significance.

The Wilcoxon Test for the Paired Case: (3)

The Wilcoxon Test has been found to be a very efficient type of non-parametric method analysis. This was the test chosen to determine whether any significant difference existed between two V/A ratios in the same person.

In this test the differences, D for each pair are calculated and their absolute values ranked in order of increasing size. The ranks are then grouped into two categories, either positive or negative. Theoretically the total of the ranks corresponding to positive and negative values should be about the same. If, however, the total of the ranks corresponding to one sign is appreciably less than that corresponding to the other, then, under the hypothesis of equal population means, the probability of obtaining by chance alone a sum of ranks less than or equal to W_1 is calculated, W_1 being the smaller of the rank totals.

The probability of getting any given sequence of signs for the difference of n pairs is given by the expression:

$$P = (1/2)^n$$

We then determine the probability of obtaining among all possible sums of ranks, one which is less than or equal to W_1. This probability is represented by:

$$(W_1 - n)$$

The value for this expression is derived from special Wilcoxon tables.

Combining the two above expressions, the probability of obtaining a sum of ranks less than or equal to W_1 (the smaller of the rank totals) is:

$$P = (\tfrac{1}{2})^n \times (W_1 - n)$$

Since we are using a two-tailed test this is multiplied by two:

$$P = 2 \times (\tfrac{1}{2})^n \times (W_1 - n)$$

Tables IX and X list the calculations used to determine the probability of rank sum differences for Twenty Diabetic subjects who had two vessel pairs in their conjunctival photographs.

TABLE IX

V/A RATIOS OF TWO VESSEL PAIRS IN THE SAME INDIVIDUAL SHOWING THE DIFFERENCE, EITHER POSITIVE OR NEGATIVE, BETWEEN THE TWO RATIOS IN DIABETICS.

SUBJECT	PAIR	R_1	R_2	$D = (R_1 - R_2)$
1		5.026	7.227	-2.201
2		3.889	5.531	-1.642
3		8.000	6.809	+1.191
4		1.056	6.087	-5.031
5		1.577	2.726	-1.149
6		3.058	6.703	-3.645
7		3.086	4.028	-0.942
8		9.515	5.370	+4.145
9		2.959	6.167	-3.208
10		7.350	11.889	-4.539
11		2.097	5.371	-3.274
12		1.317	3.500	-2.183
13		5.220	4.815	+0.405
14		6.744	11.657	-4.913
15		3.814	2.350	+1.464
16		5.096	7.593	-2.497
17		3.302	2.103	+1.199
18		1.857	3.114	-1.257
19		13.692	11.034	+2.658
20		13.022	15.225	-2.203

TABLE X

RANKING OF D = $(R_1 - R_2)$ FOR DIABETIC PAIRS

1	+0.405	11	-2.203
2	-0.942	12	-2.497
3	-1.149	13	+2.658
4	+1.191	14	-3.208
5	+1.199	15	-3.274
6	-1.257	16	-3.645
7	+1.464	17	+4.145
8	-1.642	18	-4.913
9	-2.183	19	-4.539
10	-2.201	20	-5.031

W_1 (+) $1 + 4 + 5 + 7 + 13 + 17 = \underline{47}$

W_2 (-) $2 + 3 + 6 + 8 + 9 + 10 + 11 + 12 + 14 +$
$15 + 16 + 18 + 19 + 20 = \underline{163}$

The Probability of obtaining a sum of ranks less than or equal to 47 is given by:

$$P = 2 \times \frac{(W_1 - n)}{} \times (\tfrac{1}{2})^n$$

$= 2 \times (15,506 / 1,048,576)$

$= .015 \times 2$

$= .030$

If (W_1 - n) is greater than 30 (the limit of the Wilcoxon tables) then an alternative method must be applied. For large n, W_1 (the smaller of the rank totals) satisfies an approximately normal distribution with mean:

$$m = \frac{n(n+1)}{4}$$

and standard deviation:

$$= \sqrt{\frac{n(n+1)\quad(2n+1)}{24}}$$

Tables XI and XII show the calculations used to determine the probability of rank sum differences for Twenty-two Non-diabetic subjects who had two vessel pairs in their conjunctival photographs.

TABLE XI

V/A RATIOS OF TWO VESSEL PAIRS IN THE SAME INDIVIDUAL SHOWING THE DIFFERENCE, EITHER POSITIVE OR NEGATIVE, BETWEEN THE TWO RATIOS IN NON-DIABETICS

SUBJECT	PAIR	R_1	R_2	$D = (R_1 - R_2)$
1		4.000	1.070	+2.930
2		4.670	9.750	-5.080
3		2.587	5.381	-2.794
4		10.118	4.117	+6.001
5		3.479	2.966	+0.513
6		6.317	5.264	+1.053
7		1.145	3.485	-2.340
8		8.290	5.354	+2.936
9		6.680	6.786	-0.106
10		4.953	3.016	+1.937
11		13.692	4.854	+8.838
12		6.692	12.643	-5.951
13		2.203	2.586	-0.383
14		3.015	3.023	-0.008
15		2.548	2.103	+0.445
16		2.617	3.024	-0.407
17		2.626	2.450	+0.176
18		8.805	4.159	+4.646
19		5.708	2.350	+3.358
20		3.690	4.043	-0.353
21		3.266	2.178	+1.088
22		9.816	5.614	+4.202

TABLE XII

RANKING OF D = $(R_1 - R_2)$ FOR NON-DIABETIC PAIRS

1	-0.008	12	-2.340
2	-0.106	13	-2.794
3	+0.176	14	+2.930
4	-0.353	15	+2.936
5	-0.383	16	+3.358
6	-0.407	17	+4.202
7	+0.445	18	+4.646
8	+0.513	19	-5.080
9	+1.053	20	-5.951
10	+1.088	21	+6.001
11	+1.937	22	+8.838

W_1 (-) 1 + 2 + 4 + 5 + 6 + 12 + 13 + 19 + 20

= 82

W_2 (+) 3 + 7 + 8 + 9 + 10 + 11 + 14 + 15 + 16

+ 17 + 18 + 21 + 22 = 171

W_1 has a normal distribution with mean:

$$m = \frac{n(n+1)}{4}$$

$$= \frac{22 \times 23}{4} = 126.5$$

$$\sigma_w = \sqrt{\frac{n(n+1)\quad(2n+1)}{24}}$$

$$= \sqrt{\frac{22 \times 23 \times 45}{24}}$$

$$= 30.8$$

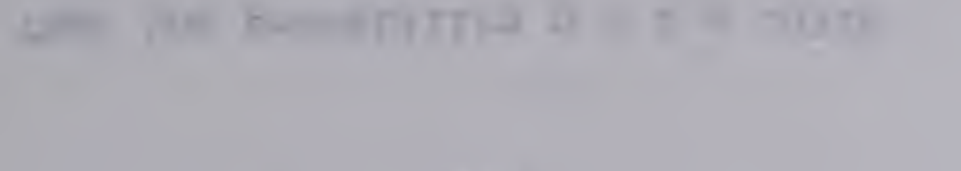
So that the probability of finding a sum of ranks less than or equal to 82 is the area under the normal probability curve to the left of:

$$z = \frac{82 - 126.5}{30.8}$$

$$= 1.45$$

Thus the probability $p = 2 \times .0735$

$$= .1470$$



APPENDIX B

CALCULATION OF MAGNIFICATION

Rather than attempt to work out, using complex mathematical formulae, the effective optical magnification that was being obtained with the optical system used, a graticle of known dimensions (the counting chamber of a hemacytometer) was photographed and subsequently measured. (Fig. XXV)

Three magnifications were desired:

(1) The resultant magnification obtained directly on the negative.
(2) The magnification obtained when the negative was projected on to a screen five feet away from the slide.
(3) The magnification obtained on the 4"x 5" prints used in this thesis.

Calculation of Magifications:

Since the graticle photographed was of a known dimension viz. one millimetre. The magnifications could easily be obtained by measuring the length of this graticle on the negatives and prints and forming a ratio with 1 mm as the denominator.

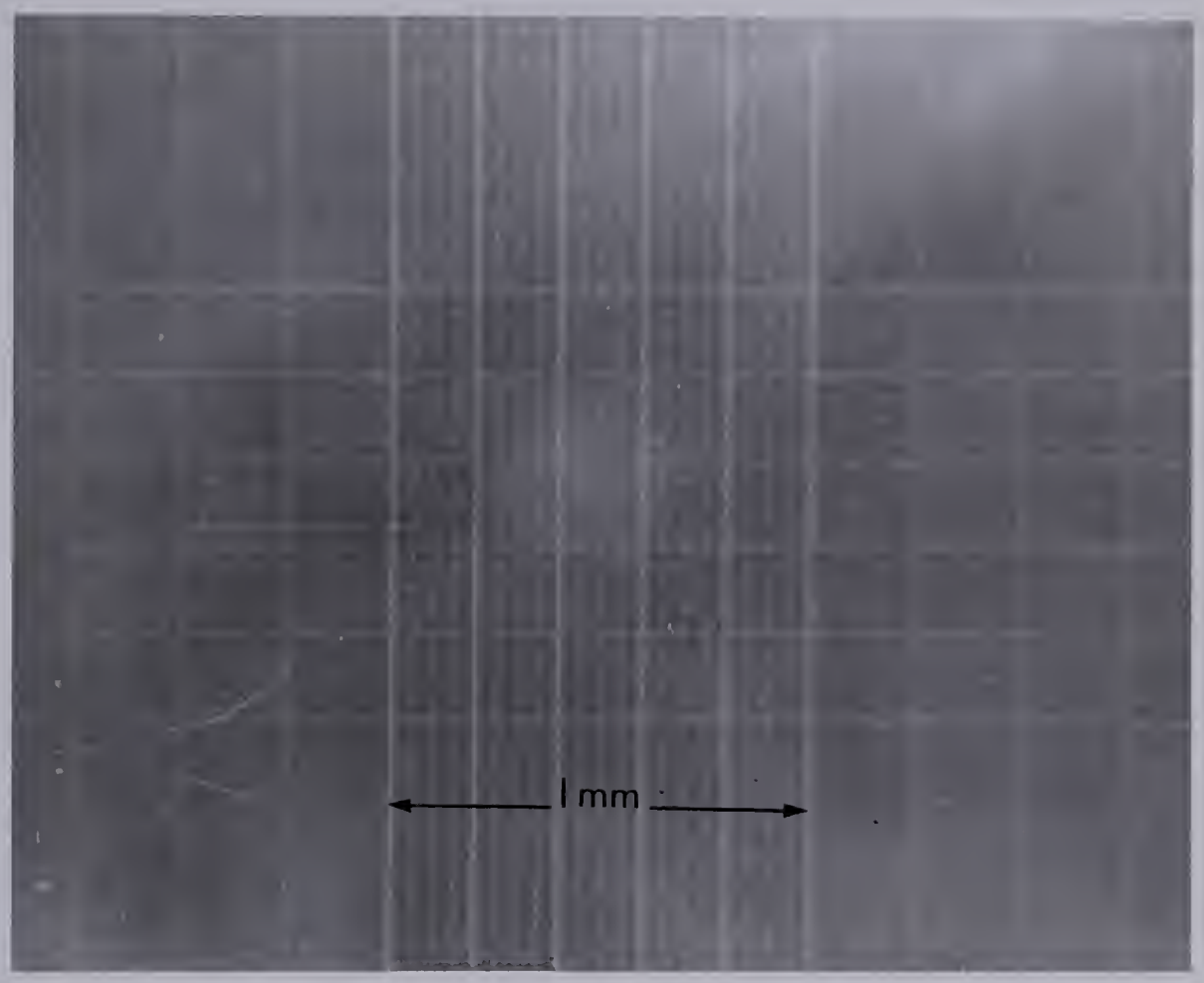


FIGURE XXV

ACTUAL PHOTOGRAPH OF GRATICLE USED FOR MAKING ESTIMATIONS OF ACTUAL OCULAR MAGNIFICATION

(1) - Magnification obtained on negative:

Length of graticle on neg. = 10 mm.

True length graticle = 1 mm.

Magnification = 10:1

(2) - Magnification obtained with projected negative:

Length of graticle on projected negative = 100 mm.

True length graticle = 1 mm.

Magnification = 100:1

= 100x

(3) - Magnification obtained on prints in thesis:

Length of graticle on print = 43 mm.

True length graticle = 1 mm.

Magnification = 43:1

= 43x

APPENDIX C

CALCULATION OF MEASUREMENT PRECISION

The precision of the observer's measurements was determined by taking ten measurements of the same venule and the same arteriole each day for ten days. Measurements of the previous day were forgotten and were kept unseen from one day to the next (Table XIII).

At the end of ten days, the means of the ten sets of measurements were pooled together and the mean and standard deviation was determined for the ten measurements on the venule and the ten measurements of the arteriole (Table XIII).

The Standard Deviation for the ten measurements on the venule was .018; that for the arterioles was .019. Since these values are very small, we get some idea of the small amount of variation from one measurement to another.

It is important to have some idea of the accuracy of one's measurements when lists of measurements are being presented.

TABLE XIII

MEAN OF TEN MEASUREMENTS OF THE SAME VENULAR AND ARTERIOLAR DIAMETERS, PERFORMED DAILY FOR TEN DAYS.

Mean Venule Diameter	Day	Mean Arteriole Diameter
2.36	1	1.03
2.35	2	1.07
2.32	3	1.07
2.35	4	1.05
2.33	5	1.06
2.37	6	1.07
2.33	7	1.07
2.37	8	1.10
2.33	9	1.06
2.36	10	1.08
2.35	Mean	1.07
2.32-2.37	Range	1.03-1.10
.018	S.D.	.019

$$\text{Where S.D.} = \sqrt{\frac{(x - \bar{x})^2}{n-1}}$$
